THE BRITISH VETERINARY JOURNAL

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EDITORIAL

VETERINARY SURGEONS IN PUBLIC LIFE

One of the compensations for the toil and sweat of a veterinary surgeon's life is the variety of his task, scene and contact with his fellows. He may work long and at unusual hours, he may become tired and exhausted physically and mentally, but unlike many other walks of life it is rare to find one who " watches the clock " or is a victim of boredom. Each day, one might almost say each hour, brings something new, a different task, a change of scene or contact. He is continually on the move ranging over a circle of the countryside which has a radius of many miles. His clientele is not restricted to one particular class, as is often the case of his friend the doctor, but it can be said to comprise a good cross section of the population in his district. He is welcome at the manor and in the cottage.

It is not surprising therefore that he acquires a great knowledge of men and affairs and becomes a veritable repository to which one can refer when in search of information or advice on a wide variety of topics which may be of personal importance or of general concern to the community.

In his work a veterinary surgeon has to depend greatly upon his powers of observation and deduction and these faculties, when applied to the ordinary things of life, should make him one of the best informed in his area; yet the majority live their lives wrapped up in their jobs and pay little attention to local affairs.

(The August issue of The British Veterinary Journal was published on August 1)

This is a matter of regret. The excuse of being too busy at their own work is often made, but it is a lame apology. A competent man is generally a busy one, and a busy man can always find time to do that extra job, so the above excuse is applicable only to the incompetent and lazy. When veterinary surgeons neglect to take an active interest in the world around them, in charitable, social or local government affairs, they miss much in life and they are not acting to the best interest of their profession, of the community, or indeed of themselves.

Periodically, discussion on the status of the profession is revived, and occasionally, as in recent times, it runs in spate. It would appear that some consider that the possession of a professional qualification entitles one to an assured social recognition and that higher rank may be attained by the addition of further tokens. There are some who think that the appellation "Vet" compromises their dignity, and there are others who think that to assume the prefix Dr. would automatically admit to a higher and more honourable place.

Such arguments are preposterous. The status of a profession depends upon the conduct of its individual members; it may vary in different regions or areas and it is assessed by the measure of public esteem. One way in which a veterinary surgeon may gain the esteem and respect of his fellow citizens is by taking an active interest in public affairs. We would like to see more veterinary surgeons taking their place in local government, on the executive committees of social and welfare societies, and in the magistracy.

The great majority of people never come into contact with a veterinary surgeon in his vocational life. If he is active in other spheres, then the circle is widened and he can command great personal regard and gain esteem and recognition for his profession.

BRITISH VETERINARY ASSOCIATION CONGRESS, 1953

Once again the venue is Aberdeen, and the Sassenach veterinary surgeons are to storm the granite city in search of intellectual food and stimulation. The programme is a good one from the academic point of view, and the social side bids fair to equal or surpass all previous efforts. The Aberdonians are a hospitable people despite current rumour, which, be it whispered, they relish and secretly propagate.

They are intensely proud of their city and its ancient University, which has done so much to enhance scientific knowledge and spread the cult of the humanities throughout the world. No doubt memories of the last Conference to be held in Aberdeen are still cherished by many who participated at that time, and we are sure that on this occasion many of the visitors will not be strangers, for they will return knowing that their welcome and entertainment will be great.

Reuter

MISS MARIE DELFOSSE, M.R.C.V.S.

When veterinary surgeons gain high renown in a field outside the purely professional sphere they bring credit to their profession, and this is especially so when the task demands great skill and courage.

Miss Marie Delfosse did this at the recent International Horse Show held at the White City, London, when she piloted her horse *Fanny Rose* to supreme honours in the Queen Elizabeth II Cup, and in competition with horsewomen of international renown.

Miss Delfosse and her sister Martha have achieved great fame in Show jumping competitions and we are proud that they are veterinary surgeons. We deem it an honour to place on permanent record in the veterinary Press a portrait of Miss Delfosse and her great horse *Fanny Rose*.

XUM

BRITISH VETERINARY ASSOCIATION

ABERDEEN CONFERENCE, 1953

Programme

N.B.—All meetings will take place in the Music Hall unless otherwise stated.

SUNDAY, SEPTEMBER 6th

8.0 p.m. "At Home," given by the North of Scotland Division, B.V.A., at the Caledonian Hotel.

MONDAY, SEPTEMBER 7th

10.0 a.m. Civic Welcome by the Hon. the Lord Provost of Aberdeen (Rev. Professor John M. Graham, M.A.), followed by Opening of Congress by the Lady Tweedsmuir, M.P.

10.30 a.m. Opening of Exhibition of Surgical Instruments, Drugs, Books, etc., by the President, B.V.A.

2.30 p.m. *Paper:* "Recent Advances at Weybridge," by Dr. A. W. Stableforth, M.R.C.V.S., D.V.S.M., Weybridge. Opener: Mr. D. L. Hughes, F.R.C.V.S., DIP. BACT. Chairman: Professor A. Robertson, M.A., PH.D., B.SC., M.R.C.V.S., F.R.I.C., F.R.S.E. Recording Secretary: Mr. L. G. Donald, B.SC., M.R.C.V.S.

8.0 p.m. Civic Reception and Dance, given by the Lord Provost and Corporation of the City of Aberdeen in the Beach Ballroom.

TUESDAY, SEPTEMBER 8th

10.0 a.m. *Paper:* "Pig Housing and its Relation to Health," by Mr. D. S. Soutar, A.R.I.B.A., A.A. DIP., A.R.I.A.S., North of Scotland College of Agriculture. Openers: Mr. D. F. Oliver, B.SC., M.R.C.V.S., and Dr. D. Luke, M.R.C.V.S. Chairman: Mr. H. M. Wilson, M.R.C.V.S. Recording Secretary: Mr. M. Maclellan, M.R.C.V.S.

10.15 a.m. Ladies' Guild Annual General Meeting.

12.15 p.m. Films, shown by Mr. D. D. Ogilvie, B.SC., M.R.C.V.S.

2.30 p.m. Demonstration (Methods of Pig Housing) at Craibstone Farm and elsewhere, arranged by Mr. D. S. Soutar, A.R.I.B.A., A.A. DIP., A.R.I.A.S. (For members only).

2.30 p.m. Ladies' Excursion, to Paradise Woods and Alford.

8.30 p.m. Ladies' Guild Dance at Northern Hotel.

WEDNESDAY, SEPTEMBER 9th

10.0 a.m. *Paper:* "Veterinary Application of Antibiotics and other Modern Therapeutic Substances with special reference to those not in common use in the United Kingdom," by Dr. S. F.

XUM

Scheidy, V.M.D., West Point, Pennsylvania, U.S.A. Opener: Mr. D. D. Ogilvie, B.SC., M.R.C.V.S., Manchester. Chairman: Dr. W. R. Wooldridge, M.SC., M.R.C.V.S. Recording Secretary: Mr. G. A. M. Sharman, M.R.C.V.S.

12 noon — Meeting of 1952-53 Council, B.V.A.

1.0 p.m. — Glasgow Veterinary School Alumnus Association Annual General Meeting and Lunch, Caledonian Hotel.

2.30 p.m. — Congress photograph.

3.0 p.m. — Annual General Meeting, B.V.A.

7.0 for 7.30 p.m. — Annual Banquet at Northern Hotel.

THURSDAY, SEPTEMBER 10th

10.0 a.m. — *Paper:* "Some Aspects of Veterinary Dermatology," by Mr. S. Jennings, M.R.C.V.S., Glasgow. Opener: Mr. E. F. Lewis, M.R.C.V.S. Chairman: Mr. A. J. MacLennan, B.SC., M.R.C.V.S. Recording Secretary: Mr. James A. Brown, M.R.C.V.S.

1.45 p.m. — Congress Excursion to Deeside. Golf Competition, Bowling, etc.

6.30 for 7.0 p.m. — Royal Dick Alumnus Association Annual General Meeting and Dinner: Northern Hotel.

From 8.30 p.m. — Informal Social Evening, with some entertainment, at the Northern Hotel.

FRIDAY, SEPTEMBER 11th

10.0 a.m. — *A Symposium of Short Papers:* (*a*) Dr. J. G. Campbell, F.R.C.V.S. "Some Aspects of Research Work at the Poultry Research Centre, Edinburgh." (*b*) Mr. J. A. Crichton, M.B.E., M.A., B.SC. "Experiments with Identical Twin Calves." (*c*) Dr. K. L. Blaxter. "Muscular Dystrophy." (*d*) Dr. W. S. Gordon, C.B.E., M.R.C.V.S., F.R.S.E., and Mr. J. H. Taylor, M.R.C.V.S. "Antibiotics in Animal Feeding-stuffs." Chairman: Mr. A. Thomson, M.R.C.V.S. Recording Secretary: Mr. J. Suttie, M.R.C.V.S.

2.30 p.m. — Visit to Rowett Research Institute by permission of Dr. D. P. Cuthbertson, F.R.S.E., and Dr. A. T. Phillipson, M.A., M.R.C.V.S. Clinical demonstration at V.I.O. Laboratory at North of Scotland College of Agriculture, arranged by Mr. P. L. Shanks, B.SC., M.R.C.V.S. (Members only).

2.30 p.m. — Ladies' Excursion to Crathes Castle.

8.30 p.m. — President's Reception and Dance at the Northern Hotel.

SATURDAY, SEPTEMBER 12th

10.0 a.m. — Closing Meeting of Congress, followed by First Meeting of 1953-54 Council.

GENERAL ARTICLES

NEWCASTLE DISEASE (FOWL PEST) IN SIERRA LEONE AND SOME OBSERVATIONS ON THE USE OF LEDERLE WING WEB (MODIFIED LIVE VIRUS) VACCINE

By JOHN D. BIRKETT, M.R.C.V.S.
Director of Veterinary Services, Sierra Leone.

It was suspected that the disease which causes such a heavy mortality amongst the poultry of Sierra Leone was Newcastle disease; the experiment outlined below has proved fairly conclusively that this is indeed the case. In spite of the fact that native poultry owners gave the assurance that the disease was so common, there was some difficulty in procuring infected birds and it was some weeks before we were able to obtain two adult birds which were in the opinion of all who were familiar with the disease, in the first stages of the malady under investigation.

Two hens were brought to the Veterinary Laboratory on March 21 with symptoms that had first been observed 48 hours previously. They were inclined to stand about with slightly ruffled feathers and with a hunch-back appearance. The cornea was opaque and both birds had difficulty in keeping their eyes open. At this stage the appetite was only slightly impaired but anorexia was later complete. The following day a respiratory gurgling and rattle developed, accompanied by an audible clamping of the beak and a nodding of the head. On this day also a slight whitish diarrhœa was seen but this did not become watery nor did it have an offensive smell. In both birds general malaise increased and the respiratory gurgling gradually became worse. One bird died on the evening of the 23rd and the other on the morning of the 24th. Post-mortem examinations revealed several well-defined and slightly necrotic ulcers in the proventriculus and in the intestines. The spleens of both birds were removed immediately after death and stored on ice.

On March 24 both spleens were emulsified in Soronsen's buffer solution and two susceptible birds were infected, each receiving half of the mixture. One of these was an eight-month-old pure Light Sussex cock, and the other was a four-month-old pure Rhode Island Red pullet. Another similar pullet was left uninoculated and was introduced as a control. Unfortunately, during the night the inoculated pullet escaped or was stolen and was not seen again. On the 26th the Light Sussex cock was ill and exhibited similar symptoms to those shown by the two natural cases of the disease and here also the diarrhœa could not be described as extreme. The bird died on the morning of the 30th and post-mortem examination revealed petechial hæmorrhages in the heart, congested lungs, soft, swollen and friable liver, inflamed proventriculus and hæmorrhagic inflammation of the large intestine, inflammed cæcum and ulcerated

XUM

valves. The uninoculated bird, which had been in contact, showed symptoms on the fifth day, and after exhibiting the typical disease syndrome it died, showing lesions similar to those described above.

On March 30 the spleen of the Light Sussex cock which had succumbed to the disease that day was used to challenge one Rhode Island Red cock, one Rhode Island Red hen and one Rhode Island Red pullet, all of which had previously been inoculated with Lederle Wing Web (modified live virus) vaccine on March 3. A susceptible Light Sussex cock was inoculated with the remaining quarter of the spleen and it died five days later after showing typical symptoms. Post-mortem lesions were characteristic. The three immunised birds have remained healthy up to the time of writing—over two months later.

This experiment proves the disease causing the heavy mortality amongst the poultry of Sierra Leone to be Newcastle disease or fowl pest and that fowls protected with Lederle Wing Web (modified live virus) vaccine are capable of withstanding a strong dose of natural virus 27 days later which indicates that a considerable immunity is established.

Difficulties have been encountered during the initial use of this vaccine in mass immunisation campaigns amongst native fowls. Ten adult Rhode Island Red birds were inoculated and no deaths occurred but all showed malaise and inappetence on the fourth or fifth days, and although this rarely lasted for more than 48 hours, egg production was suspended for at least five weeks. Thirteen four-month-old birds (eleven pullets and two cockerels) were inoculated of which two died eleven days later. There were no distinctive post-mortem lesions and no helminths or coccidia were found. Six six-week-old chickens were inoculated and two died, one two days after inoculation and the other nine days afterwards. Again there were no distinctive post-mortem lesions and blood smears showed no bacteria and the intestinal contents were negative for coccidia and worm eggs.

Field experience shows that a considerable mortality can follow vaccination amongst native-owned poultry unless the birds are very healthy which unfortunately is rarely the case. However, in Sierra Leone the losses from Newcastle disease are so enormous that it is confidently expected that in spite of these drawbacks the vaccine will in time be accepted by native poultry owners. It is also hoped to study the duration of the immunity induced by the vaccine.

Summary

It has been shown that the disease responsible for the present heavy mortality amongst the poultry of Sierra Leone is Newcastle disease or fowl pest.

It has been demonstrated that the Lederle Wing Web (modified live virus) vaccine confers a strong initial immunity but the duration of this is not yet known.

Vaccination may cause death in some birds up to four months and laying birds will cease production for at least five weeks.

It seems probable that because the disease is so prevalent in Sierra Leone the vaccine will eventually be accepted by native poultry keepers in spite of the

mortality which usually follows the inoculation of their birds, which almost invariably are unthrifty and frequently suffer from coccidiosis and helminthiasis.

Acknowledgment

I would like to acknowledge the help of Mr. S. W. Johnson, Laboratory Assistant, during the experiment.

THE EXCRETION OF REDUCING CORTICOIDS AND NEUTRAL STEROIDS IN THE URINE OF COWS WITH PARTURIENT PARESIS*

By R. B. HOLCOMBE

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PARTURIENT PARESIS or "milk fever" has been studied and discussed extensively, but up to the present moment there have been no reports published of studies on steroid excretion in the urine of cows in this disease, in pregnancy and in the puerperium. In the course of investigations into adrenal cortical activity in cattle it was decided to examine urine samples from cows in the conditions mentioned above. In order to obtain a better picture of adrenal cortical activity it was decided to utilise both reducing corticoid and neutral steroid estimations. A preliminary report of the results of these investigations is presented below.

Material

The material consisted of urine samples collected from cows suffering from parturient paresis. The cows showed the classical symptoms and responded to treatment with calcium borogluconate injections. Collections were made from apparently normal cows in the last month of gestation, immediately after calving and 4 to 8 weeks after calving.

The urine collections were made in the Ambulatory Clinic of this Department, from cows of the Swedish Red and White Breed (S.R.B.). The collections were made in the months of January to May, generally before 12 noon. During this period the cows were stall fed.

In view of the method of collection and the nature of the cases single urine samples only were obtained. This material may be considered as a random sample.

* This work was carried out under a scholarship from the Animal Health Trust, England.

Methods

The single urine samples were received at the College within two or three hours of collection, and stored in a refrigerator until analysed. No preservative was used. As a rule analysis was carried out within 24 hours, but in a few cases analysis was delayed until 24 to 48 hours after collection.

As no 24-hour urine samples were available under the above-mentioned conditions, and steroid values are liable to show significant hourly differences, results are expressed as concentration per unit-volume urine.

The neutral steroid fraction was extracted and estimated by the Zimmerman method as described by Meschaks (1948). In this case results are expressed as milligram-equivalents of androsterone per litre of urine corrected to specific gravity 1035.

The urinary reducing corticoid fraction was extracted and estimated by the method of Heard and Sobel (1946), largely as modified by Sprechler (1951). Results were expressed as milligram-equivalents of DOCA per litre urine.

Results

Group of Cows	Corticoids (mg./litre)		Neutral Steroids (mg./litre)	
	Mean	Range	Mean	Range
1. Normal, non-pregnant milking cows (4 to 8 weeks *post-partum*) (8 cases)	4.0	2.5- 4.9	19	17-23
2. Cows in the *last month of pregnancy* (12 cases)	8.2	6.1-13.5	45	35-51
3. Normal cows *within 48 hours after parturition* (8 cases) ...	5.1	3.5- 6.3	36	30-47
4. Normal cows within 48 to 120 hours after parturition (4 cases)	4.4	3.1- 5.9	30	19-43
5. Cows with "classical" *paresis puerperalis,* within 48 hours of parturition (14 cases)	1.4	0.3- 2.1	14	8-18

Discussion

The pituitary-adrenal system may be conveniently divided into three components. The first component consists of the peripheral tissue cells (liver, muscle, brain, skin, kidney, udder, etc.). Environmental change acting on these cells initiates the cycle of events leading to activation of the pituitary-adrenal system. The second component is the pituitary which interprets the changes in the peripheral tissue cells and elaborates appropriate amounts of trophic hormone. This trophic hormone acts upon the third component of the system, namely, the adrenal cortex, accelerating the secretory activity of the latter to meet the needs of the peripheral tissues for cortical hormones (Sayers and Sayers, 1948). In this paper the results of studies of the excretion of reducing corticoids and neutral steroids are reported, and these are presumed to consist largely of

metabolites of adrenal cortical hormones, and so the " third component," or the adrenal cortex, is the chief subject of discussion here.

I. Non-Pregnant, Milking Cows

These must be used as " normal " material; post-puerperal, non-milking, non-pregnant cows would in general be sterile or otherwise abnormal. Efforts were made to collect the urine only from cows with previously normal histories. It is interesting to note that the excretion of neutral steroids in cows is lower than that in healthy bulls with normal sexual functions of the same breed.

II. High Pregnant Cows

The results from the cows in the last month of gestation are considerably higher than those from normal, non-pregnant, milking cows, that is up to 3x greater neutral and corticoid excretion. This can be interpreted as considerably increased adrenal cortical activity during the later stages of pregnancy, and is comparable with the situation in women. Several authors have described a rise in the excretion of adrenal products in the urine of late pregnancy, up to 3x or 4x that of non-pregnant women (Devis, 1950; Burrows, 1943; Jayle, 1951; Parviainen, 1951). Similar results have been obtained in sheep during studies of " 17 ketosteroid " excretion in pregnancy (Whitten, 1943). The concept of pregnancy as a low-grade, gradually-increasing stress culminating in parturition has been stated by several authors (Selye, 1949; Sayers and Sayers, 1948; *et al.*). In 1951 Garm suggested from a consideration of histological and blood examinations that there also appears to be an increase in adrenal cortical activity in cattle during late pregnancy. This is confirmed by my results. He also suggests that gestation can be regarded as a stage of adaptation in the General Adaptation Syndrome corresponding to the concepts of Selye, Sayers and Sayers, *et al.* This is in agreement with the results presented here, in which I show a very great increase in adrenal cortical activity in late pregnancy. No other authors appear to have touched on this problem except Sandberg (1949), who shows an increased rate of thymus gland involution during pregnancy in cattle, which would seem to be in harmony with my results.

III. Recently-Calved Cows

These cows show a higher level of urinary corticoids and neutral steroids than non-pregnant, milking cows, and this level can be interpreted as a lowering of adrenal cortical activity after parturition. This is a parallel result to that obtained in women (Jayle, 1951).

The excretion of these adrenal cortical products falls off in the time immediately following parturition to a " normal level " for non-pregnant, milking cows, as can be seen to some extent from the results presented. This is confirmed by the results obtained by Moberg from blood morphology examinations in our laboratories.

IV. Cows with Parturient Paresis

The results from cows suffering from parturient paresis show a urinary excretion of reducing corticoids which is less than one-third of that in normal

XUM

cows in the same stage *post-partum*. The difference in the case of urinary neutral steroids is less, but nevertheless marked. This can be taken to imply a very greatly lowered activity of the adrenal cortex in this disease, which could be considered adrenal cortical exhaustion. Strikingly similar low adrenal cortical activity in association with coma is seen in the stage of exhaustion in Addison's disease in man. A similar picture from a hormonal point of view has been observed in women with eclampsia (Devis, 1950).

The stage of lowered adrenal cortical activity seems to exist before the clinical appearance of obvious symptoms of parturient paresis, as one urine sample was obtained from a cow about seven hours before the onset of paresis symptoms, and this showed a low level of reducing corticoids and neutral steroids.

As stated above, no other hormonal investigations into parturient paresis in cattle have appeared in the literature available. However, indirect confirmatory evidence of lowered adrenal cortical activity is to be found in the increase in blood ketones (Hayden, 1928) and the slight lowering of protein values (Wilson, 1932) in this disease. Pathological anatomical observations in cases of parturient paresis were made by Garm (1950), and he found an increase in hypophyseal and adrenal size. However, he states that this evidence does not definitely indicate hyper- or hypo-activity of the adrenal cortex (Garm, 1951, p. 299). However, when this observation is considered in connection with the results of analysis of urinary steroids, it can be concluded that there is hypo-activity of the adrenal cortex in bovine parturient paresis. In addition, examinations of the blood picture in this disease have been carried out by Garm (1951) and Moberg (1953), but although direct deductions of adrenal cortical activity from these appear to be open to some doubt, the results of the latter worker do not appear to conflict with those given here.

The ætiology of bovine parturient paresis has been the subject of very much discussion, and of a unique volume of hypotheses. A discussion of all this would appear to be beyond the scope of this paper, as in any case reference can be made to the review papers of Hibbs (1950) and Garm (1952).

In normal cows there is a large increase in adrenal cortical activity during pregnancy, which corresponds with the concept of pregnancy as a long-acting stress culminating in parturition. After parturition the urinary excretion of corticoids and neutral steroids falls, and eventually returns to the non-pregnant state. In parturient paresis this fall in excretion is very much greater, and is so impressive that it may be compared with total exhaustion (as in Addison's disease), with depletion of adrenal cortical reserves. This may be regarded as a typical adrenal cortical response to severe stress, as described, for example, by Sayers and Sayers (1948). The adrenal cortical exhaustion in this case probably follows closely on the onset of high udder activity. So it would appear that the sudden switch-over to high udder activity which follows milking requires an extremely large supply of adrenal cortical hormones, especially as parturient paresis is primarily a disease of heavy-milking cows, and its incidence has risen in parallel with the development of the bovine udder. Thus one may assume

XUM

that the adrenal cortical exhaustion depends in the first place on this sudden onset of high udder activity. An additional role of unknown extent is played by other predisposing factors, e.g., sudden meteorological changes. Yet another unknown factor is the role of heredity. As there has been phenotypic selection almost entirely on milk production for very many cow generations, it could be suggested that hereditary factors may play some role in the ætiology of this conditions. Thus " weak endocrine constitution " (Lagerlöf, 1950) in those cows repeatedly subject to the disease comes into the question.

The onset of the adrenal cortical exhaustion seems to be so sudden that there is no time for a Stage of Adaptation, as envisaged in Selye's (1949) General Adaptation Syndrome, to occur, but the onslaught of the stress is so sudden that the Stage of Exhaustion is reached directly and rapidly. This stage is dangerous to the life of the cow. Early appropriate treatment leads to complete and rapid recovery as in Addison's disease in man, but treatment given too late cannot revive the totally-exhausted adrenal cortex, and death may follow. This corresponds with the picture of stress given by Sayers and Sayers (1948) *et al.* Adrenal cortical depletion may be demonstrated in all cases of stress followed by adaptation to the stress and restoration of adrenal cortical reserves, or exhaustion and death (Sayers and Sayers, 1948).

Air insufflation of the udder depresses the blood supply and reduces the function of the udder, and thus a complete and rapid exhaustion of adrenal cortical reserves is avoided, allowing time for adrenal cortical mobilisation, that is by adrenal cortical stimulation and adaptation to increased secretion. The adrenal cortex is capable of very rapid response to stimulation except in cases of complete exhaustion (Thorn and Forsham, 1950). The action of calcium therapy in parturient paresis is difficult to explain as pure substitutional therapy. " It seems that the drain of blood calcium for the production of colostrum is not the primary cause of milk fever. . . . It is more likely that the cause is the inability of the regulatory system to meet this sudden demand through mobilisation " (Hibbs, 1950). Thus it would seem that calcium therapy exerts *inter alia* some stimulant action on the regulatory system of calcium metabolism.

It can be expected that the treatment of parturient paresis with ACTH and corticoids will support these findings.

Conclusions and Summary

(1) Cows in the last month of pregnancy show high adrenal cortical activity, which can be regarded as an adaptation to the stress of pregnancy.

(2) Cows after calving show a falling excretion of adrenal cortical products and a return to " normal " levels after several days.

(3) Cows with parturient paresis show a very low excretion level of urinary reducing corticoids and neutral steroids, i.e., symptoms of extreme cortical exhaustion. So parturient paresis appears to be a disease associated with adrenal cortical exhaustion, which possibly should respond to adrenal cortical stimulants, or cortical hormones, if treated before complete adrenal cortical exhaustion has occurred.

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STUDIES ON THE BIONOMICS OF THE PREPARASITIC STAGES OF DICTYOCAULUS VIVIPARUS WITH REFERENCE TO THE SAME IN THE ALLIED SPECIES IN SHEEP "*D. FILARIA*"

By K. N. SOLIMAN

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Introduction

FARMERS and stock breeders have learned through experience and without a real knowledge of the mode of operation, certain useful procedures in combating parasitic diseases. An example of such a practice is exemplified by the system of sheep grazing evolved by farmers in the North of England. They consider that a flock of sheep must not be left grazing in the same area hearing the same church bells ringing three Sundays running. From our present knowledge of the development of the free-living stages of *Dictyocaulus filaria* we can give the explanation—the development of the first stage larvæ to the infective stage needs about seven days. Such practices, although not as scientifically exact as they should be, are the result of observations of successive generations.

To consider scientifically the extent to which factors may lead to the increase or decrease in the infective parasitic forms on pasture, a sound knowledge of the epidemiology of the parasite in question is essential. In turn this can only be achieved and perfected through a real understanding of the bionomics of the infective larvæ of the given parasite.

Available information on the bionomics of the lungworm of cattle seems to be limited. Very few accounts of observations are recorded in literature bearing upon *Dictyocaulus filaria;* although the two species of parasites can be regarded as related to one another, the analogy is not adequately supported by facts. Moreover, results of experimental observations on the bionomics of the one species, *Dictyocaulus filaria,* by itself is contradictory in some respects.

The object of this work was to discover more facts regarding the bionomics of *Dictyocaulus viviparus* and also to confirm certain opinions regarding the bionomics of *Dictyocaulus filaria* to elucidate the conflicting results reported by various workers.

The study followed began with the first forms of the parasites, *viz*: The eggs, and covered different aspects as will be mentioned later.

Materials and Methods

The material used for this study consisted of:

(1) *Eggs.*—These were collected from uteri of mature adult *Dictyocaulus viviparus* and *Dictyocaulus filaria* by teasing their bodies with the point of a pair of scissors in clean tap water, or in other instances from lung exudate.

(2) *First stage larvæ of both species.*—These were collected by Baermann's technique from fæces of cattle and pellets of sheep suffering from lungworm disease.

(3) *Second and third stage larvæ.*—These were nursed through their development in clean tap water and aerated by bubbling air through the water containing them by means of an air pump.

The methods and technique followed varied according to the particular investigation and a short description is given when dealing with each point.

I. The Egg

Hatching of Dictyocaulus viviparus and filaria eggs

Freshly extruded eggs of these two species contain actively motile embryos within an intact vitelline membrane.

(*a*) *Process of hatching*

In order to follow the process of hatching a number of eggs of both species of parasites were collected from lung exudate of fresh cases, washed almost immediately after collection in normal saline and put in a well slide in a warm chamber at 37° C. and observed under the microscope.

The movements of the embryos inside the eggs of both species which had the vitelline membrane still intact were restricted, and the shape of the egg shells were uniform without any distortion. In the course of 30 minutes, most of the embryos forced their heads through this intact membrane and wriggled free inside the egg shells. Now the movements of the embryos after rupturing the vitelline membrane became less restricted. The embryos were then seen to

XUM

move vigorously using the curve of their bodies, thrusting their heads or tails forcibly against the egg wall. Owing to these movements the shape of the egg kept changing and very soon a breach in the egg shell was accomplished, through which the larvæ escaped to the outside world, this breach was usually at or near one of the two poles of the egg (see Figs. 1 and 2), but sometimes it was in the middle part (see Fig. 3). Thus the process of hatching appeared to be of a mechanical nature. No dissolution or disintegration of the egg shell was observed at this phase of hatching.

This process of hatching may take place in the lungs, since the lung exudate contained unhatched eggs and first stage larvæ together.

(*b*) *Effect of various temperatures on hatchability*

In order to find out the effect of various temperatures on the process of hatching the following experiment was repeated three times with almost constant results:

Three hundred eggs of each species were collected, so as to ensure as far as possible that they were of the same age, those with the vitelline membrane still intact were selected. Each hundred eggs, after being washed in normal saline, were put in clean sterile watch glasses with sterile tap water. Each of the six watch glasses were inserted inside an empty petri dish with a wet filter paper in it to maintain a moist atmosphere. One pair of plates was left at one of the three temperatures: 8° C., room temperature of 22° C., and 37° C. The eggs were examined under the microscope every 12 hours and the hatchability recorded as a percentage. The following tables give the results:

TABLE 1

Effect of different temperatures on the hatchability of eggs of Dictyocaulus viviparus.

Time of examination	First Series			Second Series			Third Series		
	At 8° C.	At 22° C.	At 37° C.	At 8° C.	At 22° C.	At 37° C.	At 8° C.	At 22° C.	At 37° C.
After 12 hours	8%	30%	42%	10%	28%	35%	9%	30%	41%
After 24 hours	15%	43%	61%	16%	42%	55%	15%	40%	53%
After 36 hours	21%	78%	100%	22%	69%	100%	26%	68%	100%
After 48 hours	37%	100%	—	36%	95%	—	33%	85%	—
After 60 hours	40%	—	—	41%	100%	—	39%	100%	—
After 72 hours	51%	—	—	53%	—	—	49%	—	—
After 84 hours	59%	—	—	61%	—	—	58%	—	—
After 96 hours	67%	—	—	65%	—	—	70%	—	—
After 108 hours	78%	—	—	73%	—	—	81%	—	—
After 120 hours	91%	—	—	88%	—	—	89%	—	—
After 132 hours	100%	—	—	93%	—	—	97%	—	—
After 144 hours	—	—	—	100%	—	—	100%	—	—

TABLE 2

Effect of different temperatures on the hatchability of eggs of Dictyocaulus filaria.

Time of examination	First Series			Second Series			Third Series		
	At 8° C.	At 22° C.	At 37° C.	At 8° C.	At 22° C.	At 37° C.	At 8° C.	At 22° C.	At 37° C.
After 12 hours	10%	35%	45%	11%	31%	41%	7%	27%	38%
After 24 hours	17%	48%	73%	15%	49%	79%	17%	51%	57%
After 36 hours	22%	74%	100%	26%	65%	95%	23%	73%	89%
After 48 hours	35%	97%	—	31%	89%	100%	39%	89%	100%
After 60 hours	44%	100%	—	39%	97%	—	41%	100%	—
After 72 hours	56%	—	—	47%	100%	—	55%	—	—
After 84 hours	68%	—	—	53%	—	—	69%	—	—
After 96 hours	73%	—	—	69%	—	—	77%	—	—
After 108 hours	89%	—	—	81%	—	—	89%	—	—
After 120 hours	98%	—	—	93%	—	—	94%	—	—
After 132 hours	100%	—	—	100%	—	—	99%	—	—
After 144 hours	—	—	—	—	—	—	100%	—	—

From the previous tables it will be seen that the eggs of both species hatched more quickly at 37° C., a little slower at 22° C. (room temperature), and hatchability was definitely delayed at 8° C. This may be explained on the grounds that at 37° C. the embryonic activity was at its highest whilst at the room temperature of 22° C. it was still going on but not with the same velocity. At 8° C. the movements of the embryo inside, although still visible were not continuous. These results are in agreement with Daubney's (1920).

(c) Effect of bile on the process of hatching

Or Lov *et al.* (1937) stated that the bile plays a role in the process of hatching and experimenting with eggs of *Dictyocaulus filaria* he observed that the ova hatched after 24 hours in liquids containing bile whilst in distilled water hatching took place after 96 hours. With these results in mind the following experiment was carried out with the eggs of *Dictyocaulus viviparus* and *Dictyocaulus filaria.*

Bile salt (sodium taurocholate) was added to sterile water in sterile pots, to eliminate bacterial contamination in the following percentages, 1 per cent, 3 per cent and 5 per cent. In each pot 100 eggs of each species were introduced and two sets of each series were prepared. One was left at room temperature at 22° C. and the second series was left in the incubator at 37° C. Examination was carried out every 24 hours. With each series an additional control pot containing 100 eggs in sterile water without any bile was prepared. The results of this experiment are given in Tables 3 and 4.

XUM

TABLE 3

Effect of bile on the process of hatching of Dictyocaulus filaria eggs.

	Hatching at room temp. 22° C.				Hatching at 37° C.			
Time of examination ...	1% bile	3% bile	5% bile	control	1% bile	3% bile	5% bile	control
After 24 hours	32% hatched	37% hatched	35% hatched	37% hatched	48% hatched	50% hatched	52% hatched	51% hatched
After 48 hours	98% hatched	95% hatched	100% hatched	100% hatched	100% hatched	100% hatched	100% hatched	100% hatched
After 96 hours	100% hatched	100% hatched	—	—	—	—	—	—

Observations of the actual hatching process in both these experiments only confirms the view that the process is mainly a mechanical one achieved by the efforts of the fully developed larvæ inside. No dissolution or disintegration of egg shells were observed and the work of Or Lov *et al.* was not confirmed.

TABLE 4

Effect of bile on the process of hatching of Dictyocaulus viviparus eggs.

	Hatching at room temp. 22° C.				Hatching at 37° C.			
Time of examination ...	1%	3%	5%	control	1%	3%	5%	control
After 24 hours	43% hatched	45% hatched	45% hatched	47% hatched	58% hatched	60% hatched	61% hatched	60% hatched
After 48 hours	100% hatched	98% hatched	99% hatched	99% hatched	100% hatched	100% hatched	100% hatched	100% hatched
After 96 hours	—	100% hatched	100% hatched	100% hatched	— —	— —	— —	— —

(*d*) *Where hatching of eggs of Dictyocaulus viviparus normally takes place*

In the course of this study while examining fæces samples for confirmation of diagnosis of parasitic bronchitis, eggs of *Dictyocaulus viviparus* were, on rare occasions, observed along with larvæ in the fæces collected from the rectum, in two out of 49 cases. This was observed mainly by floatation technique using concentrated sodium chloride solutions and when the samples of fæces were examined immediately. This suggested the desirability of finding out the exact spot where hatching of the eggs usually takes place. From the 49 cases that were examined in this study that succumbed to parasitic bronchitis samples were collected from the rumen, abomasum, duodenum, jejunum, colon and rectum. Cases from which such samples were collected were those that were killed, so to evade the time factor which may obviate the results. Datas are given in Table 5.

FIG. 1
Larva of *D. viviparus* forcing its way out by both head and tail ends through one pole.

FIG. 2
Larva of *D. viviparus* forcing its way out by its head through one pole.

FIG. 3
Larva of *D. viviparus* having forced its way through middle part of the egg.

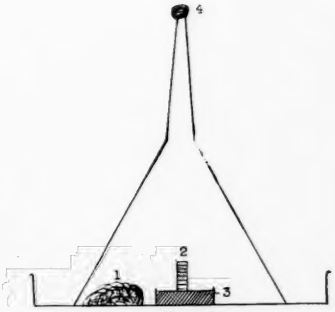

FIG. 4
Apparatus used for testing migration of larvæ.
1. Cotton-wool soaked with boiling water.
2. Graduated slide.
3. Sand.
4. Plastercin.

(*Article by Soliman, page* 364)

XUM

TABLE 5

Results of examination of fæces samples collected from different spots of intestinal tract of 20 cattle that were killed suffering from parasitic bronchitis.

Case No.	Age Y.	Age M.	Breed	Results of examination expressed per gram of fæces: Rumen	Abomasum	Duodenum	Jejunum	Colon	Rectum
ADH 1	—	8	S	8E	11E	10E	19E	70L	120L
ADH 7	—	6	J	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
ADH 17	3	—	A	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
ADH 18	3	—	A	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
ADH 22	3	—	A	Neg.	Neg.	Neg.	Neg.	5.3L	10L
ADH 23	4	—	A	Neg.	Neg.	Neg.	Neg.	10L	12L
ADH 25	3	—	A	5E	9E	11E	38E	95L	150L
ADH 27	—	8	S	Neg.	14E	19E	50E+15L	120L	210L
ADH 35	3	—	A	39E	91E	83E	90E+11L	250L	800L
ADH 37	3	—	A	11E	46E	57E	110E+21L	312L	600L
ADH 38	—	8	G	Neg.	8E	11E	15E+ 5L	108L	175L
ADH 40	3	—	A	75E+3L	82E+1L	79E	110E+43L	246L	460L
ADH 41	3	—	A	Neg.	43E	51E	50E+31L	148L	256L
ADH 42	3	—	A	3E	18E+7L	23E+6L	48E+26L	139L	312L
ADH 43	3	—	A	Neg.	20E	27E	31E+ 3L	108L	219L
ADH 44	3	—	A	5E	11E	14E	45E+ 2L	198L	342L
ADH 46	1	—	J	Neg.	10E	27E	40E+ 5L	157L	285L
ADH 47	3	—	J	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
ADH 48	3	6	A	8E	12E	17E	29E+10L	65L	106L
ADH 49	3	6	A	11E	14E	16E	27E+21L	80L	127L

S—Shorthorn; J—Jersey; A—Ayrshire; G—Guernsey; E—Eggs; L—Larvæ.

From the previous table it will be seen that ova could be constantly discovered as far down as the jejunum. No eggs were observed in any of the 20 samples collected from the colon of the 20 cattle selected. Eggs were usually unhatched down to the duodenum. Only in one instance from an Ayrshire cow were larvæ seen as well. It has already been observed that only a small percentage of eggs hatch in the lungs. The small number of larvæ thus liberated are so widely distributed in the large quantity of ingesta, that failure to discover them in the upper part of the alimentary canal is understandable.

Or Lov *et al.* (1937) trying to determine the exact spot where hatching of eggs of *Dictyocaulus filaria* in sheep took place, examined samples of ingesta from different sections of the intestines (two samples from abomasum, five from the small and three from the large intestines).

Ova were only detected in samples from anterior sections of the intestines and larvæ were recovered only in samples taken from the colon.

These results observed with *Dictyocaulus viviparus* eggs, agree in general with those of Or Lov *et al.* (1937). The fact that first stage larvæ could be observed in lung exudate is in support of those exceptional instances where larvæ were observed in the anterior section of the intestines.

The bile was shown not to exercise any appreciable effect on hatchability

of eggs of both species in vitro. Particles of food in the four stomachs and the anterior part of the intestine probably assist the embryos to effect a breach and liberate themselves from the egg shell.

II. The Bionomics of the Preparasitic Stages of Dictyocaulus viviparus and Dictyocaulus filaria.

(a) Testing of various media for development of larvæ of both species

In order to determine the best medium for culturing the larvæ of both species, the following three media were tried: Tap water, river water, and water containing fæcal washings. Two sets of 100 first-stage larvæ of each species were placed in the media to be tested and results were recorded every 24 hours, expressed as percentage of dead larvæ.

TABLE 6

Testing for favourable media for development of larvæ of Dictyocaulus viviparus.

Time of exam.	Tap Water		River Water		Water containing fæces washings	
	At 8° C.	At 22° C.	At 8° C.	At 22° C.	At 8° C.	At 22° C.
24 hrs.	all alive	all alive	all alive	1% dead	all alive	5% dead
48 hrs.	all alive	all alive	all alive	15% dead	11% dead	37% dead
72 hrs.	all alive	2% dead	9% dead	51% dead	35% dead	89% dead
96 hrs.	all alive	12% dead	17% dead	100% dead	66% dead	100% dead
120 hrs.	5% dead	56% dead	33% dead	—	91% dead	—
144 hrs.	29% dead	95% dead	51% dead	—	100% dead	—
168 hrs.	39% dead	100% dead	91% dead	—	—	—
192 hrs.	47% dead	—	100% dead	—	—	—
216 hrs.	69% dead	—	—	—	—	—
240 hrs.	81% dead	—	—	—	—	—
264 hrs.	92% dead	—	—	—	—	—
288 hrs.	100% dead	—	—	—	—	—

TABLE 7

Testing for favourable media for development of larvæ of Dictyocaulus filaria.

Time of exam.	Tap Water		River Water		Water containing fæces washings	
	At 8° C.	At 22° C.	At 8° C.	At 22° C.	At 8° C.	At 22° C.
24 hrs.	all alive	all alive	all alive	3% dead	all alive	7% dead
48 hrs.	all alive	all alive	all alive	21% dead	all alive	14% dead
72 hrs.	all alive	3% dead	7% dead	45% dead	10% dead	43% dead
96 hrs.	all alive	17% dead	19% dead	90% dead	23% dead	81% dead
120 hrs.	3% dead	21% dead	27% dead	91% dead	31% dead	100% dead
144 hrs.	21% dead	44% dead	32% dead	100% dead	41% dead	—
168 hrs.	51% dead	65% dead	54% dead	—	69% dead	—
192 hrs.	67% dead	91% dead	85% dead	—	100% dead	—
216 hrs.	89% dead	100% dead	100% dead	—	—	—
240 hrs.	95% dead	—	—	—	—	—
264 hrs.	100% dead	—	—	—	—	—

XUM

These observations showed that in river water and in water containing fæces washings, the larvæ perished at a room temperature of 22° C. in four days in the case of *Dictyocaulus viviparus* and in five to six days in the case of *Dictyocaulus filaria*. In tap water that was not changed, this was complete in seven days in the case of *Dictyocaulus viviparus* and in nine days in the case of *Dictyocaulus filaria*. At the lower temperature of 8° C. death occurred more slowly in the same media.

This experiment showed that for the development of these larvæ oxygen is essential and that bacterial contamination or putrification of organic material which consumed the oxygen, death was hastened.

It therefore seemed important to ensure the oxygen supply and eliminate bacterial, protozoal or organic contamination and accordingly the technique followed for ensuring normal development was to change the tap water every day and to bubble air continuously into it. This was carried out at a room temperature of 22° C. for seven days to ensure that all larvæ reached the infective stage. Then the water was changed and larvæ kept at 8° C.

(*b*) *Thermotropism and skin penetration*

Khalil (1922) put forward the suggestion that a positive thermotropism might be a characteristic of skin penetrating larvæ. He had in mind the positive reaction to heat exhibited by Ancylostoma larvæ when the point of a hot needle was applied to the under surface on a watch glass containing these larvæ in water. Cameron (1923) testing *Bunostomum trigonocephalum* and *Nematodirus spathiger*, Schwartz (1925) working with *Bunostomum phlebotomum*, Baudet (1925) investigating *Trichonema spp*, and Monnig (1930) trying larvæ of *Trichostrongylidæ* all showed that positive thermotropism characteristics are shared as well with non skin-penetrating larvæ.

Cameron (1927) remarked that hosts of skin-penetrating forms are not grass eaters. Monnig in 1930 showed that Strongyloides species which infect ruminants penetrate the skin of these hosts.

In connection with the infective preparasitic stage larvæ of both *Dictyocaulus filaria* and *Dictyocaulus viviparus* there is no data regarding their thermotropic characteristics except that of Daubney (1920). He experimented only with the infective third stage larvæ of *Dictyocaulus filaria* on the shaved skin of a lamb and three guinea pigs and came to the conclusion that they do not appear to be able to penetrate the unbroken skin.

EXPERIMENT 1

Thermotropism : This was tested in two different ways with both *Dictyocaulus filaria* and *Dictyocaulus viviparus* third stage larvæ.

(1) *Khalil's test* (1922). Applying the hot end of a dissecting needle to the undersurface of a watch glass containing the larvæ and watching the effect under a dissecting microscope. The larvæ near the source of heat showed increased activity but they were not at all attracted by it.

(2) *Monnig* (1930) was doubtful whether the movement of the larvæ towards the source of heat could be attributed to their activity or to convection

currents caused by the heat. He devised another method for the test which was applied to the larvæ of both species. Two glass tubes of 15 cm. long by 7 mm. in diameter were filled with water containing the larvæ of both species, corked at both ends and fixed horizontally. A thin copper wire was wound round the middle part of each tube with one end of the wire projecting at right angles. The larvæ inside the tubes were watched by a hand lens while heat was applied to the projecting piece of the wire. Care was taken to apply such heat so as not to cause convection currents in the water. The larvæ situated nearest to the warm part displayed increased activity but the remaining ones outside the zone where heat was applied were not at all attracted towards the warm middle section.

EXPERIMENT 2

Skin Penetration : Sheathed and ex-sheathed third stage larvæ of both *Dictyocaulus viviparus* and *filaria* were used in testing for this phenomenon. The experiment was carried out in two ways :

(1) Using freshly removed abdominal skin of young mice and guinea pigs.

(2) By applying the larvæ to the skin of live animals.

The hair in both cases was removed by means of clipping so as to avoid skin abrasions that might result from shaving.

(1) *Freshly isolated skin :*

(*a*) Applying Goodey's method (1922), four cork " rafts " were prepared. The centre aperture of two were covered with the abdominal skin of a mouse and the other two with that of a guinea pig. The " rafts " were floated in four separate containers in Ringer Locke solution kept at 37° C. Approximately 2,000 sheathed larvæ were put on one " raft " and the same number of the ex-sheathed larvæ on to another. This process was repeated with the other two " rafts " but this time using the larvæ of *Dictyocaulus filaria.* These were transferred by means of a fine pointed pipette. The water containing the larvæ was allowed to evaporate and the " rafts " were left for six hours. No evidence of the presence of larvæ was found in any of the four cases.

(*b*) The same experiment was repeated again with a little modification. The four pieces of skin were tied on to the top of each of four thistle funnels. The end of each funnel was attached to a small test tube by means of a rubber tube similar to Baermann's apparatus. The funnels were filled with Ringer Locke solution before fixing the pieces of skin. Care was taken not to allow any air bubbles between the skin and the solutions in the funnels. Each funnel was then kept in a water jacket at 37° C. Several thousand of each species of larvæ both in the sheathed and in the ex-sheathed state were applied to the middle part of the skin. The water containing the larvæ was allowed to evaporate and then after a period of six hours had elapsed the solutions in the four attached test tubes were examined and found to be negative in each case.

In both these experiments the eight pieces of skin which were used were fixed in hot 70 per cent alcohol, cleared in lacto-phenol and then examined under the dissecting microscope. No larvæ were observed in any of the eight pieces of skin.

XUM

(2) *Testing on skin of live animals*

Two mice and two guinea pigs were used in this experiment. One square inch of hair on the abdominal region was clipped. Three thousand larvæ in both the sheathed and ex-sheathed forms were applied to the clipped area. The water containing the larvæ was allowed to evaporate. The animals were killed five days later. The mesenteric lymphathic glands, livers and lungs were examined by means of glass compressors as used for detecting trichinella and also by means of mincing these organs and hanging the material on Baermann's funnels filled with normal saline and kept at 37° C. Mesenteric glands, livers, and lungs of the two guinea pigs and the two mice gave negative results.

A search was carried out on the part of skin concerned by fixing it in hot 70 per cent alcohol, and clarifying in lacto-phenol. This failed to reveal any larvæ in the skin of the four animals.

(*c*) *Phototropism*

Daubney in 1920 showed that the larvæ of *Dictyocaulus filaria* favour darkness for their migration and that they have been observed to ascend four centimetres perpendicularly during eight hours of darkness. He found that direct sunlight is unfavourable to migration and that strong sunlight repelled the larvæ in the direction of the culture medium providing there is sufficient moisture. The following experiments were conducted with the three stages of both species in relation to this phenomenon.

EXPERIMENT 1

(1) *First-stage larvæ*

Larvæ of both species were obtained by Baermann's method four hours after collection of fæces samples directly from rectum to ensure that they were just at the beginning of the first stage period. Approximately 200 of each species were smeared on a slide so as to cover an area at least an inch long. Sufficient water was added to prevent dryness. Using a black metal sheet half of the smeared area was covered leaving the remainder exposed to daylight. The slides were kept in the horizontal position inside a petri dish over the glass stand of the dissecting microscope so that examinations could be made with the least disturbance. After two hours the metal sheet was removed. In the case of both species of larvæ the majority were observed to have collected in the part exposed to light of the oval area.

The same experiment was then repeated using 0.1 grams of fæces of high larval count from both cattle and sheep freshly collected from the rectum. Knowing the larval count already an amount was weighed out containing 100 larvæ of each species. These samples were spread over two slides with 0.1 c.c. of tap water on an area similar to the above. The same technique was employed using the black metal sheet. On examination after two hours' exposure to light both species of larvæ showed a powerful degree of phototropism. Most of the larvæ were observed to have collected on the half of the slide that was exposed to daylight.

If these slides were left for 24 hours in the same moist chamber, larvæ of

XUM

both species showed no response to light, being evenly distributed between light and dark areas. It was noted that by this time the larvæ had reached the second stage.

(2) *Third-stage larvae*

The experiment was repeated with third stage larvæ of both species but there was no response to light. The larvæ appeared to have been inactivated by the sunlight.

The experiment was again repeated with the third-stage larvæ of both species with a little modification. Two small glass tubes of 5 mm. diameter and 15 centimetres long were filled with water and 1,000 larvæ added. The tubes were then held horizontally and each inserted into the cardboard covering of a thermometer with one-third of each tube exposed to direct sunlight. After three hours' exposure larvæ were found uniformly sedimented on the bottom of each tube and had clearly been unaffected by the light.

It appeared that only the first-stage larvæ of both species exhibits phototropic characteristics. This sensitivity may be a factor which assists the larvæ to leave the fæces when the fæces are freshly dropped.

(*d*) *Resistance to dryness*

Results of observations recorded by different workers on the resistance of the preparasitic stages of both *Dictyocaulus filaria* and *Dictyocaulus viviparus* are conflicting.

Guberlet (1919) stated that embryos of *Dictyocaulus filaria* reared in moist soil till they developed protective sheaths when allowed to dry and kept in a thoroughly dry state for five months, showed signs of life 15 to 20 minutes after remoistening. Daubney (1919) carried out an experiment on the third-stage larvæ of both these parasites. He observed their revival after apparently complete desiccation for 16 hours. The process of drying and remoistening was repeated four times after which some live larvæ were still observed. It was only after the fifth time of desiccation that no larvæ at all revived. The same worker in 1920 repeated the same experiment with only one difference in his technique. The larvæ were collected from the walls of a culture jar using a camel-hair brush and placing them in water on a clean slide, thus avoiding any debris. The results this time were totally different from those he got in 1919 as no larvæ revived after 16 hours' desiccation, although he kept re-examining the slide which was kept in a moist chamber for 16 hours after moistening. Daubney (1920) mentioned that Dr. Ransom informed him of the results of a similar experiment he carried out in 1918 with third-stage larvæ of *Dictyocaulus filaria*. Ransom's results of 1918 were exactly similar to Daubney's observations in 1919 as he was able to observe revival of some larvæ on the fourth time of desiccation over 16 hours.

Or Lov *et al.* (1937) tested the resistance of larvæ of *Dictyocaulus filaria* from cultures of different ages that were collected from Baermann's and smeared over a cover-glass. One-, three- and five-day old larvæ were tested. One- and

three-day old larvæ of *Dictyocaulus filaria* never revived after 24 hours' desiccation. In the case of five-day old larvæ when desiccated for 24 hours, 5 per cent revived after one hour and the figure went up to 20 per cent after 24 hours. The same author stated that desiccation for three days slowed down the revival and for five days caused still slower revival and that in the latter case one larva only revived after one hour. No mention of the actual state of exposure in the last author's experiment was given, although it seems that it is the main point to be considered in interpreting any results of such a nature.

The conflicting results obtained by various workers on this point seem to be mainly due to the variation of the conditions of exposure which in fact determine the real degree of dryness of the larvæ. So if any stable results are hoped to be recorded, the experiments must be carried out under controlled conditions. Contradiction in results may arise from causes such as:—

(1) Presence or absence of debris in water containing larvæ.

(2) Relative humidity of the surrounding atmosphere.

(3) Temperature under which experiments are carried out.

(4) Number of larvæ used in the experiment, i.e., the possibility of some seeking refuge under the aggregated bulk when dried, if a large number are used.

(5) Evenness of surface on which they are dried and the presence of cracks which might harbour a certain amount of moisture.

With all these factors in mind the following experiments were carried out:—

Experiment 1

Cultures of larvæ of both *Dictyocaulus filaria* and *Dictyocaulus viviparus* were obtained from fæces of infected animals according to Baermann's technique. These cultures were washed in clean water several times and the individual larvæ were picked out by means of fine pipette under a dissecting microscope thus ruling out the presence of debris. Well-glass slides were examined under the microscope and any showing cracks were discarded, 25 larvæ were used each time so the chance of larvæ taking refuge underneath each other was reduced.

After the slides were prepared they were left at room temperature (which was recorded), to dry. The experiment was carried out with the three preparasitic stages of both species of *Dictyocaulus* previously mentioned. The well-slides were allowed, after apparent evaporation of the water to stand on the bench for 10 minutes, thus ensuring actual dryness. They were kept in this state of dryness at four different temperatures, namely 0° C., 8° C., 20° C. (room temperature) and at 37° C. (in the incubator). Each of the four well-slides was kept inside a petri dish which was completely dry and sealed with plasticine to ensure dryness. Each of the four petri dishes was kept at one of the previously mentioned temperatures. At the beginning of this experiment slides were allowed to stay in this state for 24 hours then they were remoistened and examined every half an hour subsequently being kept in a humid atmosphere. Examination of slides was carried out over 12 hours then the next day a final

XUM

result was recorded after a further 12 hours before they were discarded. When no revival at all was observed the time of dryness was shortened until revival of some larvæ was observed. Then each of the last experiments was repeated three times to ensure stability of results.

Results of Observations on Effect of Dryness on the Preparasitic Stages of Dictyocaulus viviparus and Dictyocaulus filaria

(1) *First-Stage Larvæ*

Observations obtained from experiments carried out with the first-stage larvæ of both species showed that this stage of larvæ is extremely susceptible to the effect of desiccation, so much so, that it failed to survive three hours' complete desiccation when being kept at any of the previously mentioned four different temperatures. Not one larva revived after the addition of water even after being kept in a moist atmosphere for a further 24 hours.

(2) *Second-Stage Larvæ*

Second-stage larvæ of both species proved to be able to survive three hours' desiccation when kept in sealed petri dishes at 8° C. Larvæ of both species when kept in such a condition after desiccation at 0° C. and 37° C. all perished. At a room temperature of 20° C. only *Dictyocaulus filaria* showed signs of revival. Out of 25 larvæ, one revived one hour after moistening, thus representing a 4 per cent survival rate. This one larva, however, failed to survive another three hours' desiccation.

At 8° C. the survival rate of *Dictyocaulus filaria* was 8 per cent and that of *Dictyocaulus viviparus* was 4 per cent, and again the three larvæ that survived of both species failed to withstand another three hours' desiccation under the same conditions as before.

The survival rate up to one hour after moistening was exactly the same each time the experiment was performed and no revival of any larvæ was encountered when slides were examined at intervals up to 24 hours after moistening. Larvæ that survived desiccation seemed to loose their sheaths.

From this it seems that second-stage larvæ of both species show a little more resistance to drying than those of the first-stage and that *Dictyocaulus filaria* second-stage larvæ are somewhat more resistant to dryness than those of *Dictyocaulus viviparus,* i.e., higher percentage rate at 8° C. and by the revival after dryness at a room temperature of 20° C.

(3) *Third-Stage Larvæ*

Observations on the effect of desiccation on the third-stage larvæ of both species proved that this stage is more resistant to dryness than the first two. Not one larvæ survived 24 hours' desiccation after being moistened although observations were carried out at intervals up to 24 hours after moistening also.

After 16 hours' dryness : Larvæ of both species kept at temperatures of 0° C., 20° C. and 37° C. all perished. Of those kept at 8° C., *Dictyocaulus filaria* survival rate proved to be 4 per cent but no *Dictyocaulus viviparus* larvæ revived. The experiment was repeated three times with the same results.

After 12 hours' dryness : Larvæ of both species kept at 37° C. all perished and none whatsoever revived after moistening and keeping in the wet state for 24 hours subsequently.

At a room temperature of 20° C. the rate of revival of the three repetitions of the same experiment with *Dictyocaulus filaria* were respectively 16 per cent, 12 per cent, and 16 per cent, with an average of 14.6 per cent.

Dictyocaulus viviparus revival rates under the same conditions were 16 per cent, 8 per cent and 16 per cent, with an average of 12 per cent.

At 8° C. the results were as follows :—

Dictyocaulus filaria revival rate 20 per cent, 16 per cent and 20 per cent, with an average of 18.6 per cent.

Dictyocaulus viviparus revival rate 16 per cent for the three repetitions with an average of 16 per cent.

At 0° C. the results were as follows :—

Dictyocaulus filaria revival rate 16 per cent, 20 per cent and 16 per cent, with an average of 17.3 per cent.

Dictyocaulus viviparus revival rate 12 per cent, 12 per cent and 16 per cent, with an average of 13.3 per cent.

None of the surviving larvæ showed resistance to dryness under the same conditions for another 12 hours. The majority of the survived larvæ were observed to have lost their sheath on remoistening.

The effects of dryness on third-stage larvæ are in agreement with Daubney's (1920) observations on *Dictyocaulus filaria,* none reviving after 16 hours' desiccation, with one exception. One larva in the experiment cited here revived after this time in all three tests at 8° C.

As regards *Dictyocaulus viviparus* none at all survived this time at any of the four selected temperatures.

For a 12-hour period of desiccation a higher survival percentage was observed with *Dictyocaulus filaria* than with *Dictyocaulus viviparus.* Further the resistance to dryness in the second stage was greater than for the first stage and the infective stages of both species of larvæ were the most resistant forms of the three preparasitic stages.

An interesting feature that was observed after moistening was that the larvæ were curled in the form of a ring. This may be a natural reaction on the part of the larvæ to protect themselves against desiccation.

(e) Effect of temperature

Temperature affects the motility of the larvæ, variations either incite active movements or inhibit them.

The three different stages of the two types of larvæ were exposed to temperatures of 0° C., 8° C., 20° C. and 37° C. The maximum activity was observed in both species between 20° C. and 30° C. becoming less towards 37° C. Temperature of 8° C. and lower seemed to inhibit totally movements of larvæ of all three stages.

It was observed also that when first-stage larvæ of both species were exposed

XUM

to temperature of —5° C. they were killed in 20 minutes. Second and third stages of both species were not affected by such temperature and when they were thawed out again were found active and alive on being warmed.

The three stages of both species when put in tap water at 60° C. were almost stunned dead.

(f) Effect of moisture

For larvæ of both species to survive they require moisture. As previously shown, dryness is detrimental to these larvæ. Third-stage larvæ of both species possess a considerably greater degree of resistance to desiccation than do the earlier stages.

(g) Migration

A number of observations have been recorded on the powers of migration that certain nematode larvæ exhibit under favourable conditions. The temperature, moisture, light and type of medium are the four main factors that govern this phenomenon, and affect the species in different ways. There may also be other factors or circumstances which affect migration and explain certain anomalies. For example the work of Cameron, who in 1923 experimented with larvæ of *Bunostomum trigonocephalum* found that they do not migrate and with a little difference in the consistency of the culture medium, the same worker in 1927 showed that they do actually migrate.

There is little work apart from that by Daubney (1920) on the migration of the final-stage larvæ of *Dictyocaulus filaria*. This author mentioned that migration of the infective stage of *Dictyocaulus filaria* will occur only in an atmosphere saturated with water vapour and that water condenses on surfaces of objects in contact with the environment in which these larvæ were present.

Owing to the importance of the role that migration may exercise in connection with the epidemiology of parasitic bronchitis, the following experiments were carried out to study the factors influencing this phenomenon.

The three stages of both species of larvæ cannot swim nor can they suspend themselves in deep water. In common with other nematode larvæ they cannot migrate upwards from water or from a very wet medium as they cannot overcome the surface tension. This prevents them leaving the environment, a fact which is easily demonstrated by watching the movements of the larvæ under the microscope in a drop of water.

EXPERIMENT

Procedure of experiment

A small round pot was filled with fine sand up to ½ centimetre of its edge. The sand was then wetted with tap water and 20,000 of the larvæ in 1 c.c. of water poured over the surface so as not to leave any water visible. This avoids any influence which might result from surface tension. A glass slide divided crossways into sections of ½ and 1 centimetres was inserted upright into the sand. The sand was slightly heaped up the two sides just to the beginning of the first graduation. A glass funnel 10 inches in diameter was inverted

over the pot and the whole placed on an enamel tray. The tip of the funnel was then closed with plasticine. Pieces of cotton wool soaked in boiling water were inserted underneath the funnel (see Fig. 4). The water vapour condensed on the surface of the slide and the whole apparatus was left at room temperature (22° C.) at 9 o'clock in the morning.

Observations

Dictyocaulus filaria infective larvæ : The larvæ migrated seven centimetres in the upward direction in 24 hours. All were coiled in a ring form on the glass surface. The experiment was repeated three times with the same result every time.

When the same experiment was performed and the funnel left in a dark room for 24 hours the results were nearly the same. The larvæ in each case travelled 6½ centimetres.

Dictyocaulus filaria, first and second stages : No migration was observed whatsoever.

Dictyocaulus viviparus infective larvæ : Repeating the experiment with this species the larvæ were observed to have very slight migratory powers. They migrated ½ centimetre only in 24 hours. The experiment was repeated five times at room temperatures of 20° C., 22° C., 25° C., 27° C., and no greater migration was observed.

Dictyocaulus filaria, first and second stages : No migrations at all were observed.

From the foregoing experiments it is obvious that the contrast in the migratory powers of infective stage larvæ of the two species is very great. *Dictyocaulus viviparus* are very feeble climbers, whilst those of *Dictyocaulus filaria* are strong climbers. The one main point that could be concluded was that *Dictyocaulus filaria* larvæ proved to be active climbers. Although both species are allied yet they behaved differently under same conditions.

Epidemiologically this difference would seem to be a very important feature for it would seem that *Dictyocaulus viviparus* relies to a great extent on being carried up by the herbage as it grows. The larvæ that happen to be on the under-surface of the leaves are protected to some extent and are not washed by the rain.

If this is the true explanation then, as they are poor climbers, in dry seasons when the grass is poor one would expect to find them around the roots of the herbage. In consequence of the herbage being poor in quality and quantity it is also likely that the animal will crop more closely and be likely to get more infective larvæ, and this may account for the sudden onset of parasitic bronchitis in cattle under such conditions.

On the other hand, in wet seasons when the grass is lush, cattle are apt not to graze and the parasitic larval population is thus left round the roots. When such grass grows up, most of these larvæ will be lifted with the growth and thus will be near the tips of blades when such meadows are ready for second grazing. Larvæ thus would be available in enormous numbers and when cattle

XUM

graze such grass a sudden onset of the disease is likely to occur. The same explanation may be applied to fields on which the grass was cut for haymaking.

Summary

(1) Process of hatching ova of both *Dictyocaulus filaria* and *Dictyocaulus viviparus* is a mechanical one.

(2) Bile seems to play no role in the process of hatching as no dissolution or disintegration of the egg shell could be observed.

(3) Hatching of larvæ from ova usually takes place in the first part of the alimentary canal and is usually completed by the time the ingesta reaches the colon.

(4) Tap water proved to be a favourable media for culturing larvæ of both species.

(5) Infective larvæ of both species were neither attracted nor repelled by heat.

(6) Infective larvæ of both species proved to be non-skin penetrators.

(7) First-stage larvæ of both species exhibited actual phototropism in contrast to second and third stages which displayed none.

(8) Actual desiccation over 16 hours was detrimental to larvæ of both species. The most resistant stage is the third-stage larvæ. *Dictyocaulus filaria* infective stage proved to be more resistant to dryness than *Dictyocaulus viviparus*.

(9) Variation of temperature affected the activity of larvæ of both species. The optimum temperature range at which larvæ exhibited full activity was found to be between 20° C. and 30° C.

First-stage larvae of both species when exposed to temperature of —5° C. were killed in 20 minutes.

Second and third stages of both species remained alive at that temperature.

Temperature of 60° C. killed both species of larvæ of all three stages almost immediately.

(10) Only third-stage larvæ exhibited migratory powers. *Dictyocaulus viviparus* proved to be a feeble climber, while *Dictyocaulus filaria* proved to be a powerful one.

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NEWCASTLE DISEASE IN NIGERIA

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In January, 1951, Lindley (1951) drew attention to outbreaks of Newcastle disease in the middle Congo and along a line spreading eastwards from the Gambia, as far as the Northern Territories of the Gold Coast and Haute Volta. Severe outbreaks had previously been reported from Dakar, French Senegal (Mornet, 1950). Up to this time no report of Newcastle disease had been received from Nigeria, but owing to the presence of the disease in neighbouring territories the Veterinary Department carried out prophylactic inoculations in certain areas, using the Komarov strain of Newcastle disease vaccine obtained from the Onderstepoort Laboratories, Pretoria, South Africa. In April, 1951, however, Kirkby (1951) made a diagnosis of Newcastle disease in two outbreaks among poultry in the Eastern Region; this diagnosis was, unfortunately, not confirmed. Further outbreaks resembling Newcastle disease were reported from Ibadan and other parts of the Western Region during the period December, 1952, to February, 1953.

In view of the doubts which persisted regarding the presence of Newcastle disease in Nigeria, laboratory tests were carried out on material received from cases in the Western Region. Two mild strains of the virus were used for the tests, one from the Ministry of Agriculture, Veterinary Laboratories, Weybridge, Surrey, and one from the Onderstepoort Laboratories, the latter being obtained from viable vaccine. Both these viruses were established on embryonating eggs and the harvested fluids from various passages were used as a source of virus for the hæmagglutination test. Immune serum, also from Weybridge, was used as a positive control serum for the hæmagglutination-inhibition test.

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XUM

By means of this test, Newcastle disease was diagnosed in a fowl from Benue Province in January, 1953. During the same month a virus was isolated from a case in the outbreak at Ibadan described below, by inoculating bone marrow from an affected bird into a White Leghorn cock. From this cock the virus was introduced on to the chorio-allantoic membrane of embryonated eggs, with bone marrow used again as inoculum, and when well established it was harvested in the allantoic fluid. The presence of the virus of Newcastle disease in the harvested fluid was confirmed by means of the hæmagglutination-inhibition test and by inoculation into susceptible birds. Further confirmatory tests were made by inoculating this virus into birds protected against the Weybridge and the Onderstepoort virus. The virus from Ibadan failed to infect these birds and there was therefore no doubt of its identity with the virus of Newcastle disease.

From November, 1952, clinical cases and post-mortem material were brought in from outbreaks of poultry diseases in the Western Region to the Division of Animal Husbandry and Health, Department of Agriculture, University College, Ibadan, for laboratory examination. Four cases of suspected fowl cholera were received on December 6 and certain pens of pullets within the University College flock exhibited similar symptoms from December 22. Two sixteen-month-old R.I.R. pullets died on the night of December 22 and post-mortem lesions were suggestive of cholera. Between the above date and January 3, 1953, several birds died in their houses overnight, these peracute cases apparently heralding the main outbreak.

Out of a total of 356 laying pullets and cockerels in the pens involved in the outbreak, 201 birds (193 pullets, 8 cockerels) died, or were slaughtered for control or diagnostic purposes. Out of the remaining 155 birds, 59 recovered, while 96 were observed not to be clinically affected.

Despite the fact that the poultry farm extends over eight acres and holds about 2,000 birds on free range (this total includes 1,200 growers and chicks), the outbreak remained confined to two groups of three-pen units and a $1\frac{1}{2}$-acre range, representing three main foci of infection. Although egg production ceased abruptly in affected pens, there was no drop in production in the remainer of the laying flock. Recovered birds, on an average, came back into full lay over a period of about four to eight weeks.

The outbreak reached its peak between January 26, 1952, and February 7, 1953, with occasional losses occurring till February 15, 1953.

All birds in the College flock, including those affected in the outbreak described, had been vaccinated at one month of age with the Onderstepoort vaccine. The vaccine had to be shipped by air from South Africa, and it is possible that some of the batches received had lost their immunising properties. Losses were, in actual fact, 25 per cent in the laying flock, but only 10 per cent of the whole flock. Control measures included the removal of sick birds from infected pens, and, alternatively, the transfer of whole pens of birds on to new ground, after having first removed any birds suspected of infection. This latter method of control appeared to give by far the most satisfactory results.

XUM

As the bacteriological examination at Ibadan was negative, samples of bone marrow and spleen from infected birds were sent to the Veterinary Department laboratories at Vom where the bacteriological tests were also negative. The virus of Newcastle disease was isolated from these samples and has been described above. Samples of serum from Ibadan were also examined; of 17 samples from non-suspect birds all proved negative, whilst 20 of 21 of the samples from survivors of the outbreak were positive.

Positive results were also obtained with samples of serum collected from four birds which had recovered during an outbreak in the Western Region.

Nearly all the affected birds were pullets and cockerels of imported breeds, aged one year to 18 months and bred at Ibadan. Except for peracute cases there was extreme prostration and inappetence, followed by sneezing, coughing and choking; these latter symptoms occurred from the second to the third day. A profuse, yellowish-green diarrhœa was usually observed about the first or second day. Thick, tenacious, greyish-white discharges were present in the pharynx in the birds showing respiratory distress, but discharges from the nares were rare. Blood or croupous debris was not observed in these discharges. Conjunctivitis and occasionally opacity of the cornea were seen and nervous symptoms appeared in about 10 per cent of cases and were usually in those birds where respiratory symptoms were either slight or absent. Most deaths occurred between the second and sixth day. Recoveries were rare in cases where nervous symptoms were present, or where respiratory symptoms had been severe.

Gross lesions were not observed in all peracute cases, but in those in which they could be seen the post-mortem picture presented resembled that of the acute cases, except that enteritis was usually either slight or absent. Acute cases were characterised by the presence of sero-sanguinous exudates in the thoracic and abdominal cavities, fibrinous pleuritis and pericarditis, and occasional cloudiness of the air-sacs. The lungs showed varying degrees of inflammation, including grey, hepatised areas in prolonged cases. Hæmorrhagic areas were frequently observed in the trachea and œsophagus, with bloody exudates in some cases in the larynx. Heart lesions included endocarditis and hæmorrhages in the myocardium, especially around the coronary vessels. Inflammatory changes in the liver, spleen, kidneys and intestines were the most conspicuous lesions. Greyish-white necrotic, pin-point foci were present under the liver capsule in advanced cases. Hæmorrhages in the proventriculus and in the fat surrounding the gizzard were accompanied, in most cases, by varying degrees of enteritis, often hæmorrhagic, in the duodenum and ileum. A "greying" of the eyes was a common lesion. No gross lesions of the brain were observed. From post-mortem lesions it would appear that the virus was viscerotropic in nature.

*Microscopic Lesions.**—*Liver* (Plate 1): Typical architecture lost. Extensive intracellular œdema. Sinusoids dilated and empty. Extensive vacuolation in hepatic cells, but nuclei mostly normal and showing no displacement. Consider-

* Microscopical findings were confirmed by Dr. W. D. Silvera, Head of the Department of Pathology, Faculty of Medicine, University College, Ibadan.

XUM

able infiltrations of red blood corpuscles and mononuclear cells. Extensive granulocytosis. Kupffer cells prominent. *Kidneys* (Plate 2A and 2B): Glomeruli—normal in size, lumina appearing patent, but some swelling of epithelium of Bowman's capsules, some signs of urates and red blood cells in glomerular spaces. Tufts—filtration apparently taking place, but endothelial cells swollen. Tubules—markedly swollen, inner margin of cells of epithelium ill-defined and granular, with karyolysis and karyorrhexis in some cells, considerable debris in lumina, including desquamated epithelial cells, cellular changes in both convoluted tubules and loops, generally advanced cloudy swelling and some necrosis. Interstitial tissue—widespread congestion of blood vessels, the predominent microscopical lesions are massive tubular changes.

Slight œdema with no inflammatory reaction was seen in the brain of a bird showing nervous symptoms.

It is impossible to apply rigorous control measures in the remote areas of Nigeria, but the present policy of prophylactic immunisation, especially on Government farms and where there are co-operative poultry farms, is advisable. Lloyd (1952) has stated in England that serious consideration of a vaccination programme depends upon the likelihood of the disease becoming endemic. Jezierski (1950) has reported good results from vaccination in the Belgian Congo, and Alexander (1953) considers the Komarov strain of Newcastle disease vaccine to be of considerable value in South Africa, and that it should be useful in Nigeria.

The entry of Newcastle disease into Nigeria presents a serious threat to the country's poultry industry. Although the standard of poultry husbandry is low, the hardy little West African chicken forms an integral part of the Nigerian's household and agricultural economy. There are no rearing costs, and the housewife can assure for herself and for her children a steady source of income by the sale of her birds at the local markets. Indigenous poultry seem fairly resistant to infection with *Pasteurella* and *Salmonella,* but there can be little doubt that they are highly susceptible to the virus of Newcastle disease, as witnessed in the devastating outbreaks in the Gold Coast during 1950-51 (Annual Report, 1950-51).

Summary

During 1950-51 outbreaks of Newcastle disease in territories bordering on Nigeria and in the Middle Congo were reported. The disease was suspected in Nigeria in 1951 and an outbreak at Ibadan during December, 1952, is described in detail. This outbreak was confirmed by laboratory diagnosis in February, 1953. Prophylactic measures and the difficulties encountered in applying an effective large-scale control programme in Nigeria are discussed.

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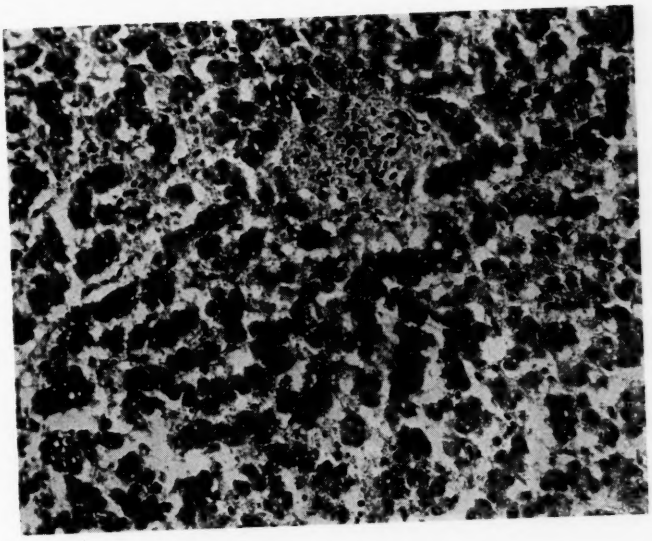

FIG. 1

FIG. 2(*a*)

FIG. 2(*b*)

(*Article by Hill, Davis and Wilde, page* 381)

XUM

XUM

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CARLO RUINI AND "L'ANATOMIA DEL CAVALLO"

By K. M. DYCE, B.Sc., M.R.C.V.S. and R. H. A. MERLEN, M.R.C.V.S.

FROM the time of the compilation of the Hippiatrika, in the reign of Constantine the Seventh, to the sixteenth century, the veterinarian had to rely upon the writings of the Greek and Roman empirics for the rudiments of his art. Throughout the succeeding centuries there had been no worth-while contribution to veterinary literature, for the Arabs, in spite of their reliance on cavalry for the fulfilment of their enterprises, had confined themselves to repeating almost verbatim the old veterinary treatises and had added nothing of their own.

For the veterinary art, 1598 marks the end of the dark ages, for it was in this year that Carlo Ruini published in Bologna the famous work entitled "L'Anatomia del Cavallo et suoi Rimedii."* It was this work that was destined to be the mainstay of veterinary anatomy for well over a century and was the spark that touched off the renaissance of veterinary studies in Europe.

It is impossible to over-emphasise the importance of this work in the development of veterinary thought, and, while it has always attracted the attention of the veterinary historian, this interest has been restricted in the last forty years to the question of its authorship and to the views it contains on the circulation. Little attention has been paid to the most significant part of the book, namely the anatomical description. In view of the importance of this work it is felt that a detailed analysis of the anatomy should be available and while having this task in hand, we propose, as a preliminary, to examine once more the vexed point of the authorship for despite the many examinations of this question no clear-cut opinion has materialised and, as Leclainche puts it: "la question de Ruini est ouverte."

The difficulty arises in the first place because so little is known of Ruini's life. The labours of Count Ercolani and others have brought to light a few details from which a very faint and imperfect portrait of the man emerges. This amounts to little more than that he was born in Bologna in about 1530 and that he was assassinated in 1598, a victim of the political troubles of the times. His father was a man of wealth and position, a jurist, a senator and a Gonfaloniere di Guistizia, and Carlo Ruini succeeded in time to all his father's dignities. In all this there is nothing that gives a clue to his interest in veterinary matters and

* While we have consulted the first edition for Ruini's Preface we have mainly used the second Venetian edition of 1599. By a curious error the title of the first edition is misspelt "L'Anotomia . . ."

XUM

the explanation for this must be sought in his preface to the first edition, where he speaks of his love for horses and his distress at the suffering caused by lack of knowledge of their ailments.

Ruini's work, as is well known, consists of two quite separate parts, the Anatomy and the Medical treatise. Ruini admits that his interest lay in the study of the diseases and ailments of the horse but he observes that as long as so little was known of anatomy and physiology there could be little progress in the recognition and treatment of disease; with commendable energy he threw himself into acquiring this fundamental knowledge. Ironically, after he had completed the studies which were to be preliminary to his main task, he failed to appreciate the value of what he had done with the result that his treatise on the infirmities is little better than many other contemporary works and if his fame were to depend on this composition he would only be remembered as one among many. Despite this he must be regarded not only as the father of veterinary anatomy but as the father also of veterinary science, for it is " L'Anatomia del Cavallo " that provided the sure foundation on which his successors built.

The work was published a few days before his death and had an immediate and continued success, running through many editions in Italian and in translation within a few years. Its success is understandable for at that time there was in Italy both an enthusiastic interest in anatomy and a strong tradition of " Cavalarice " and horse-mastership.

We have already indicated that we do not propose to examine Ruini's anatomy critically at the moment but to investigate the question of authorship, determining if we are able, the traces of contemporary and earlier inspiration which may or may not be evident in the text.

Ruini wrote in his mother tongue and this, together with his legal training, resulted in a style that is lucid and unaffected, and in descriptions that are straightforward and free from jargon. The work is moreover, free from pseudo-metaphysical argument and this is in sharp contrast to the writings of so many contemporary scientists who found themselves constitutionally incapable of considering any topic without engaging in philosophical dialectics.

In " L'Anatomia," Ruini follows the Galenical tradition and deals in succession with the head, the thorax, the abdomen, the genital organs and the extremities: in each region he describes first the bones and then the muscles and soft parts so that his account is a compromise between the truly topographical and the strictly systematic approach. Each of the five parts is illustrated by numerous wood-cuts which shew the relationships of the organs: there is in addition an appendix of seven plates illustrating the skeleton, the vessels and the muscles of the whole animal. These superb illustrations, the first illustrations that had ever appeared of the anatomy of the horse, were alone sufficient to earn the work the reception it so justly deserved.

Ruini's text is a straightforward exposition of the anatomical facts and reference to his descriptions of such particular parts as the ear, the larynx and the fœtal heart bears witness to the excellence and detail of his work. While certain other passages are less explicit and contain occasional perplexities and error,

XUM

Ruini's great merit is that as far as he was able, he described what he saw rather than what he believed should be there—as Bruni has so aptly observed "L'Anatomia del Cavallo" could well be subtitled "cose viste." The physiological descriptions are comparatively few in number and are less easy to follow than the purely anatomical passages. This is particularly true of the account of the circulation of the blood for while at times his text appears to show that he had grasped the fundamental principles of the circulation he expresses himself with such ambiguity that it is impossible to be certain of his true meaning. The fact that he was well versed in the physiological thought of his day seems to have made it impossible for him to free himself of the current misconceptions and his account is an uneasy compromise between what he was inclined to believe and what he had been taught was true.

Attempts to interpret this part of the text have led to debate and bitter controversy: some claim for him the merit of recognising the true nature of the circulation while others deny him all understanding of it. An excess of enthusiasm had led to the setting up of tablets commemorating his discovery, but it is more than doubtful if this claim can be substantiated and perhaps the fairest commentary is that of Bruni who recently declared: "Ruini, no more than his contemporaries, was the discoverer of the circulation of the blood: but among those who truly contributed to the solution of this problem, he occupies a place of honour although not, perhaps, the principal one."

The illustrations are a vital part of the work and as with Vesalius in human anatomy, we have for the first time clear and well executed woodcuts which assist materially in the comprehension of the text. These illustrations are in sufficient detail to allow the finer points to be discerned and have obviously been drawn from actual dissections. While inaccuracies can be discovered here and there and the correct proportions have not always been preserved they are an outstanding achievement and are obviously the work of an excellent draughtsman though not we believe the work of a great artist. These illustrations are in fact so remarkable that many critics have sought to see in them the work of some of the great masters—Leonardo da Vinci, Titian or the elder Carracci.

That these illustrations are most commonly ascribed to Leonardo is presumably because of an obscure statement of his that he had produced, or intended to produce, a work on horse anatomy: it is just possible that this work was completed and that it may have disappeared together with the vanished notebooks, but on the other hand Leonardo often gave promise of writing works on a variety of subjects that do not appear to have been executed. Moreover, from the surviving notebooks and drawings it does not appear that his interest in the anatomy of the horse took him beyond making a study of proportions and the examination of the flayed carcass, for, with trivial exceptions, his sketches of horse anatomy are readily identifiable as preliminary studies and cartoons for his major works such as "The Battle of Anghiari" and the Sforza statue. Schmutzer has made a careful study of the proportions of the Leonardo drawings and compared them with Ruini's and finds it impossible to believe that the latter can have been Leonardo's work. A detailed analysis is not necessary, for a very

XUM

superficial comparison is sufficient to show that Ruini was not indebted to Leonardo for his illustrations. The plates in the " Anatomia " often show the horse in rigid and unnatural positions, and Leonardo, even when illustrating anatomical structure and topography, always insisted that form should be moulded by symmetry and artistic rightness. Even when all allowance is made for the different techniques employed in the preparation of the Ruini tables, and for the intervention of the block-maker, it would seem quite impossible to lay Ruini under a debt to the great artist as Bayon, Leclainche, and others have attempted to do : Bayon has even gone so far as to see the hand of Leonardo in the ornamentation with which the illustrator has embellished certain of the plates. Ascriptions to Titian, the elder Carracci and the others have not been pressed for there is no evidence to support their case. The Ruini illustrations appear to us to have been produced by a skilled craftsman rather than by a great artist and assuming, as seems reasonable, that Ruini did not execute them himself we suggest that they were produced by a craftsman working under his direction : in this case we may perhaps see the influence of a north German style in certain of the plates, notably those of the first book. Whatever their origin they are very excellent and many are fully equal to the plates illustrating the " Fabrica " of Vesalius which set a new standard of anatomical illustration.

Just as attempts have been made to deny originality to the plates so there have been efforts to deprive Ruini of the credit of composing the text. It has been said that the lost notebooks of Leonardo da Vinci provided the basis for the text, presumably on the grounds of the same obscure statement to which reference has been made above, but the lucid and straight-forward manner of the text is very far removed from the inspired and disconnected jottings in which Leonardo recorded his observations, and it is doubtful if any editor could have worked them up into so integrated an account.

" L'Anatomia " and " L'Infermita " show so wide a knowledge of human anatomy and medicine that many people have been induced to believe that the author was a medical man. It must, however, be remembered that in those times an interest in medicine such as would be unusual to-day was common enough among men of liberal education and the dilettanti played an important role in the development of the science of the Renaissance and later, particularly in Italy. The medical influence is thought by some to come from Vesalius and it has even been asserted that Vesalius was the author. This suggestion can be scouted immediately for Schmutzer has investigated Vesalius's knowledge of animal anatomy with great care, and while there is ample evidence that the great anatomist was familiar with the structure of a number of animals including the cow and dog, there is only passing reference on one or two occasions to the anatomy of the horse. Vesalius's turgid and longwinded Latin, indulging in endless repetition and hyperboles of style, is vastly different from the simple and lucid writing of Ruini whose prose was singularly free from the more tiresome and often tasteless affectations of the day. It cannot be denied, as Bruni has attempted to do, that Ruini was familiar with the work of Vesalius and a number of passages recall parts of the " Fabrica " and even at times seem to be translations of this : in

Anatomia

DEL CAVALLO

INFERMITA,

ET SVOI RIMEDII.

Opera nuoua, degna di qualsiuoglia Prencipe, & Caualiere, & molto necessaria à Filosofi, Medici, Cauallerizzi, & Mareschalchi,

DEL SIG CARLO RVINI SENATOR BOLOGNESE.

Adornata di bellissime figure, le quali dimostrano tutta l'Anatomia di esso Cauallo.

DIVISA IN DVE VOLVMI.

De quali questo Primo, in cinque Libri copiosissimamente dichiara tutte le cose appartenenti alla detta Anatomia.

Con due bellissime Tauole, vna de' Capretti, & l'altra delle cose genitali.

All'Illustrissimo, & Eccellentissimo Signor

CESARE DI BORBONE, FIGLIVOLO

del Christianissimo Henrico IIII. Re di Francia, & di Nauarra, &c.

CON LICENTIA DE I SVPERIORI, ET PRIVILEGII.

IN VENETIA, M D XCIX.

Appresso Gasparo Bindoni, il Giouane.

FIG. 1

FIG. 2

FIG. 3.

FIG. 1

The title page of a copy of the second edition in the Library of the Royal Veterinary College.

FIG. 2

Ruini's plate illustrating the fœtal membranes of the foal.

FIG. 3

Plate illustrating the muscles of the hind limb.

(*Article by Dyce and Merlen, page* 385)

addition, as Schmutzer has shown, the inspiration for a number of the plates must have come from Vesalius for the resemblances are too close to be coincidental. It is possible to admit this without accepting the suggestion that Ruini's work is a plagiarism of the "Fabrica" for it amounts to little more than Ruini's studying the anatomies of his predecessors before undertaking his own work—as a modern scientist might read previous works on his subject before making his own contribution. The division of the book and general approach to the subject which is a regional exposition exonerate Ruini from this charge. Schmutzer, however, convinced of Ruini's guilt, insists that this is a device adopted by Ruini to conceal the extent of his plagiarism of Vesalius.

His investigations of the authorship of "L'Anatomia" induced Schmutzer to give an account of an involved relationship connecting Ruini, his nephew Francini and Heroard. Jehan Heroard was a physician at the French court who had a profound interest in veterinary matters and who according to his own account intended to produce a comprehensive work on the anatomy, management and diseases of the horse. Of this ambitious design only the first volume, on the osteology was published; this was in the year following the appearance of Ruini's work and it is natural to assume a connection between the two events—it arouses for example a suspicion of piracy, particularly as Ruini was assassinated within a few days of the appearance of his book which was thus left unprotected and at the mercy of every plagiarist.

Comparison of the two works shows many points of difference in spite of their superficial resemblance: Heroard's text and illustrations are of equal merit with those of Ruini but the emphasis is differently stressed by the two authors; for example, Heroard studied the foot in considerable detail while Ruini strangely neglects this subject. The short interval of time which elapsed between the appearance of the two works is probably enough to remove the suspicion that Heroard had pirated the Italian work, for there was hardly enough time for the preparation of the copper-plates with which "L'Hippostologie" is illustrated. Heroard published only this first volume and it is probably fair to infer that he stood down in favour of Ruini whose work had already appeared and was attracting considerable notice. The appearance of the two works at almost the same time is without much doubt purely coincidental.

Francini, Ruini's nephew, later produced a French translation of Ruini's treatise on the diseases and claimed it as his own but this impudent lie is unlikely to deceive many.

Schmutzer has elaborated an involved theory connecting these three men, suggesting that Heroard, strongly influenced by Vesalius and basing his approach on the "Fabrica," completed his opus, but that the manuscript became lost in Paris during the religious troubles of May, 1588, and that it later fell into the hands of Francini. Schmutzer goes on to suggest that uncle and nephew conspired to produce the work as their own after re-arranging it to disguise its source and he carries the accusation still further by declaring that Carlo Ruini then cheated his nephew of his share of the spoils by claiming sole authorship for himself. This fantastic story taxes credulity to the utmost and while no facts

are known that definitely disprove it, there is so little evidence in its support that it is surely illogical to prefer it to the simple explanation.

Ruini was surely the author of the work that bears his name—a man of mark such as he could have had little ambition to aspire to be known as the author of a work on the anatomy of the horse unless he was in fact genuinely attracted by the subject. Moreover Carlo Ruini as a man prominent in the State, could hardly have followed his inclinations in this direction without it being publicly known and to have produced a work on anatomy without being known to be an anatomist would surely have aroused a scepticism which could not have escaped contemporary notice.

The question of the authorship has diverted attention from the study of the book itself: the closing years of the sixteenth century are remarkable for the appearance of the first anatomy of the horse in the modern style and it is with the text that we should be mainly concerned.

We are indebted to Mr. H. R. Burgess of the Anatomy Department of the Royal Veterinary College for reproducing the illustrations.

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STUDIES ON DISINFECTION OF EGGS AND INCUBATORS

II.—The Value of Formaldehyde Gas with Particular Reference to the Concentration Resulting from the Addition of Formalin to Potassium Permanganate*

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Introduction

SEVERAL workers have reported variation in the results obtained using formaldehyde as a means of disinfecting egg incubators. The more important of these reports are summarised in Table I.

* This work was carried out with the assistance of a grant from the Agricultural Research Council.

TABLE I

Formaldehyde Fumigation, using Formalin and Potassium Permanganate

Author and date	Amounts recommended (per 100 cubic feet) at normal incubator temperature and humidity		Duration of fumigation	Ports open or closed	Efficiency of method
	Formalin	Pot. Permang.			
Bushnell, L. D. (1929)	35 c.c.	17½ gm.	1 hour	Closed	Not satisfactory
Graham, R. (1932)	35 c.c.	17½ gm.	30 minutes	Open	Not satisfactory
Graham, R. (1941)	35 c.c.	17½ gm.	Three fumigations at 12-hour intervals during hatching period	Open	Not satisfactory
Marcellus, F. N. (1930)	150 c.c.	100 gm.	1 hour	Closed and machine airtight	Not satisfactory
Burton, W. H. (1946)	150 c.c.	100 gm.	20 minutes	Not stated	Satisfactory
Wilson, J. E. (1951)	150 c.c.	100 gm.	30 minutes	Not stated	Satisfactory
	35 c.c.	17½ gm.	30 minutes	Not stated	Not satisfactory
M./A. Accredited Scheme (1951)	A: 43 c.c. (1½ oz.)	28 gm. (1 oz.)	20 to 30 minutes		
	B: 84 c.c. (3 oz.)	56 gm. (2 oz.)	20 to 30 minutes		

The reports of Burton (1936) and Wilson (1951) indicate that 150 c.c. of formalin per 100 cubic feet of incubator space gave effective results. These workers did not confirm the findings of Marcellus (1930), who failed to obtain satisfactory results with 150 c.c. formalin. For comparative purposes the amount of formalin recommended in the Ministry of Agriculture's Accredited Scheme (P.S.I.P. 1951) is included.

In view of the conflicting results quoted, it was decided to re-investigate the efficiency of formaldehyde as an incubator fumigant. Special attention has been directed to the correlation of the gas concentration, obtained by direct analysis, with the bactericidal efficiency.

Methods

Clean, uncracked, newly laid eggs were contaminated with *S. pullorum* in one of the following ways:—

(1) The eggs were immersed for a few seconds in a suspension of *S. pullorum* prepared by adding 20 c.c. of an overnight broth culture to 150 c.c. distilled water: the viable count (method of Miles and Misra, 1938) was approximately 60 million organisms per c.c.; or

(2) 0.04 c.c. of undiluted broth culture was dropped on the surface of the shell of each egg.

The eggs were then placed, in groups of six or twelve, on incubator trays and stacked in the manner usually employed for incubating eggs.

Dry incubator debris, including chick down, small pieces of eggshell and dust, etc., was mixed with 20 c.c. of bacterial suspension prepared as above. Small amounts of the contaminated debris (each piece about the size of a pea) were placed on the incubator trays which also held the contaminated eggs. Duplicate sets were prepared and one tray was placed in a standard forced-draught incubator and exposed to the fumigant, whilst the second was placed for the same period of time in a similar forced-draught incubator maintained at the same temperature and humidity. In some experiments a third tray was left at room temperature. The need for adequate controls has been shown in a previous publication (Lancaster and Crabb, 1953), in which it was found that, using the method described, *S. pullorum* dies rapidly on the surface of clean eggs under normal incubator conditions.

The eggs were removed, with sterile forceps, to screw-capped wide-mouthed jars containing 200 c.c. selenite F. medium. The incubator debris was transferred to tubes of 10 c.c. of the same medium. Jars and tubes were incubated at 37° C. for 40 hours and a loopful of each streaked on desoxycholate citrate agar plates containing 1 per cent sucrose and 1 per cent salicin. The plates were incubated for 48 hours and all non-lactose fermenting colonies were examined by the rapid-slide agglutination test.

The concentration of formaldehyde gas was estimated by collecting known volumes of air (approximately 500 c.c.) from the incubator into flasks which had been evacuated to a given level of mercury. By means of a thistle funnel attached to the flask, 25 c.c. of distilled water was run into the gas sample;

XUM

this was facilitated by placing the flask in a refrigerator at —20° C. for a few minutes, the contraction rapidly drawing in the distilled water. When the distilled water had run into the flask the stop-cock was turned and the formaldehyde solution was titrated by using a standard iodemetric method*; the results are expressed in milligrams of formaldehyde per cubic foot of air. The air samples were obtained through plastic capillary tubes attached to the evacuated flasks, and samples collected from different situations inside the incubator, and also at different times. One of the incubators used in this work was rendered air-tight by means of adhesive tape and plasticine.

Results

Results of bacteriological examinations, without estimation of the gas concentration

No great attention was paid to the air-tightness of the machine, apart from opening or closing the ventilation ports. The contaminated eggs and debris were placed in the incubator, and after an interval of one hour the required volume of formalin was poured on potassium permanganate crystals in a shallow dish. In some experiments swabs were prepared according to the method of Graham and Michael (1932). The swabs consisted of pieces of sterile absorbent cotton-wool, each 1 in. by 1 in. by $\frac{1}{16}$ in., attached to lengths of sterile copper wire. These swabs were placed on the incubator trays with the eggs and fluff.

The eggs, fluff, etc., were cultured immediately after the period of fumigation; results are summarised in Table II. They are variable and in some experiments of this series *S. pullorum* has not survived, while in repeat experiments there has been a high recovery rate.

This variation in the results obtained with formaldehyde has been observed by Graham (1941) and Burton (1946). The latter author found that the efficiency of fumigation depended on the machine itself, and considered that variations under field conditions might be due to differences in the air-tightness of different incubators.

* To each flask of formaldehyde solution was added 10 c.c. N/50 iodine and then 3 c.c. of 2N.NaOH. The flask with its contents was shaken and allowed to stand for 10 minutes. Excess NaOH was neutralised with 3.5 c.c. 2N.HCl, and the liberated iodine was titrated with N/50 thiosulphate solution, using starch as an indicator. A blank titration, using a flask containing 25 c.c. distilled water only, was also made. The weight of formaldehyde in milligrams per cubic foot of air was calculated from the equation:—

$$H.CHO = I_2 = 2Na_2I_2O_5H_2O$$

The calculation was then:—

$$\frac{30}{248 \times 2} \times \frac{4.96 \text{ (i.e., grams thio in N/50 thio)} \times \text{(blank-titration reading)}}{1}$$

all multiplied by: $\frac{\text{Flask volume}}{28{,}315}$ (i.e., c.c. per cubic foot)

Results of estimations of formaldehyde gas concentration

The observations have been made in one forced-draught incubator which had been rendered air-tight with adhesive tape and plasticine. The gas concentration was related to the bacteriological result; this is shown in Fig. 1.

Each gas estimation was the mean result from three separate gas samples taken simultaneously. The bacteriological efficiency of each gas concentration figure is based on the results of the cultural examination of each of eighteen *S. pullorum* contaminated eggs. In some experiments contaminated fluff has been exposed to varying concentrations of formaldehyde.

The results shown in the figure are based on approximately 100 separate gas analyses. It will be seen that at normal incubator temperature and humidity,

TA

Formaldehyde Fumigation Pullor

Amount per 100 cubic feet		Length of exposure to gas (in mins.)	Position of incubator vents	Bacteri			ults follo ion
Formalin cc.	Pot. Permang. gram.			Eggs	Incubator I Swabs	Fluff	Eggs
42	28	60	Open				5/24
84	56	60	Open				12/72
84	56	30	Closed				4/24
42	28	60	Open	4/24			
84	56	60	Open	2/12		12/12	
147	98	60	Open	12/66			
84	56	30	Closed	2/24			

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a minimum concentration of 6.5 mg. of formaldehyde per cubic foot is required to kill *S. pullorum* on eggshell and pieces of incubator dust after an exposure to the gas for 20 minutes. The bactericidal action falls as the concentration of gas falls.

The bactericidal efficiency of formaldehyde is appreciably reduced at ordinary atmospheric humidity; thus 3.5 mg. of formaldehyde destroys 60 per cent of *S. pullorum*, whereas with a high humidity the same gas concentration kills 80 per cent of *S. pullorum*.

The mean gas concentrations obtained 20 minutes after the release, at normal incubation temperature and humidity, of 84 c.c. and 42 c.c. formalin, in a sealed and incompletely sealed incubator, are also given in the figure.

Discussion

There is apparently no published data on the formaldehyde gas concentrations obtained in incubators, and it appears that the formaldehyde concen-

XUM

tration has a direct relationship to the bactericidal efficiency. Fresh normal eggs were used in order that natural conditions may be reproduced as closely as possible, and a direct comparison with some of the published work cannot be made, since many authors have used either pieces of eggshell (Graham and Michael, 1932) or whole eggshells from which the contents have been removed (Wilson, 1951); in many cases the eggs or shells have been autoclaved before use.

Burton (1946) has drawn attention to the escape of formaldehyde gas through cracks and badly fitting doors of many commercial incubators. A completely air-tight machine has been used in our work in order to eliminate the leakage of unknown and variable amounts of formaldehyde.

The results of both series of experiments can be correlated. It will be seen

TA

migation Pullorum *Contaminated Material*

Bacteri…ults following …ion				Controls (kept for same period as fumigated material).				Number of separate experiments made
	Incubator II			Non-fumigated Incubator			At room temp.	
Flu… Eggs	Swabs	Fluff		Eggs	Swabs	Fluff	Eggs	
5/24	5/12	10/12		23/24	4/4	2/4	18/18	2
12/72	0/30	8/72		38/72	8/12	42/52	68/72	6
4/24				6/24			21/14	2
								2
12/12				3/12		12/12	11/12	1
							35/42	7
				5/24			23/24	2

numer… the number of eggs, etc., infected with Salmonellae.
…nominator = the number of eggs, etc., examined.

from Fig. 1 that 42 c.c. of formalin in an incubator which was sealed, apart from the ports being open, gave an average concentration of 4.5 mg. per cubic foot; this is below the minimum level for complete bactericidal effect. The higher strength of formalin, viz., 84 c.c., in a sealed incubator, except for the ports being open, gave an average gas concentration of 7 mg. per cubic foot, which is at the minimum level for complete destruction of *S. pullorum*. The average gas concentrations would be lower in an incubator with cracks and badly fitting doors, and it can be assumed that the closing of the ventilation ports (Table II) did not render the machine sufficiently air-tight to produce the required lethal as concentration. That this assumption is probably correct was clearly demonstrated when in later experiments the air-tightness of the particular incubator in question was tested by the generation of smoke under pressure inside the incubator; numerous cracks were then found. It appears that 147 c.c. formalin per 100 cubic feet is insufficient in such badly leaking incubators with the ventilation ports open (Table II). Many, if not all, of the results shown in

XUM

Table II were probably due to the fact that the concentration of formaldehyde was on or below the minimum level, and this in turn was due to unknown leakage of gas through cracks in the incubator and around the doors.

The results clearly support the findings of Wilson (1951), who showed that 35 c.c. formalin per 100 cubic feet was not invariably effective against *S. typhi-murium*. It has been shown in Paper I in this series, that *S. pullorum, S. thompson* and *S. typhi-murium* are similar in behaviour and susceptibility.

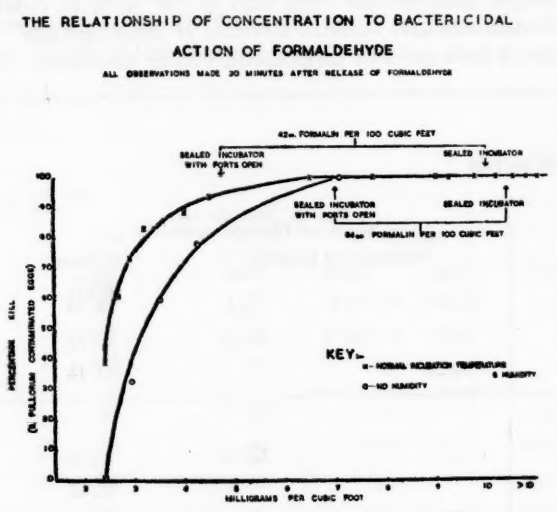
THE RELATIONSHIP OF CONCENTRATION TO BACTERICIDAL
ACTION OF FORMALDEHYDE
ALL OBSERVATIONS MADE 20 MINUTES AFTER RELEASE OF FORMALDEHYDE
42c.c. FORMALIN PER 100 CUBIC FEET
SEALED INCUBATOR WITH PORTS OPEN
SEALED INCUBATOR
SEALED INCUBATOR WITH PORTS OPEN
SEALED INCUBATOR
84c.c. FORMALIN PER 100 CUBIC FEET
KEY:-
MILLIGRAMS PER CUBIC FOOT

Fig. 1

Similarly, the present work confirms the observations (Wilson, 1951) that 150 c.c. formalin was always effective, provided the incubator was free from cracks and other sources of leakage.

Summary

(1) A method is described for the measurement of the formaldehyde gas concentration which develops in an egg incubator after the addition of formalin to potassium permanganate crystals.

(2) The formaldehyde gas concentration has been correlated with bactericidal action, using *S. pullorum* contaminated eggs and incubator fluff and debris as an indicator.

(3) A minimum level of 6.5 mg. of formaldehyde per cubic foot, in an incubator running at normal incubator temperature and humidity, is required to give complete sterilisation of *S. pullorum* contaminated eggs. These results are based on an exposure time of 20 minutes.

(4) The effect of leakage of gas from the incubator on the bactericidal action of formaldehyde is discussed.

(5) The effect of humidity on the efficiency of formaldehyde fumigation is also shown.

Acknowledgments

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A PRELIMINARY REPORT ON A RAPID FIELD TEST FOR CONTAGIOUS BOVINE PLEUROPNEUMONIA

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Introduction

The demonstration of agglutinins in the blood of animals infected with contagious bovine pleuropneumonia by Heslop (1922) indicated that the agglutination test might be of use as a means of diagnosis. He concluded that the agglutination test was superior to his complement fixation test for diagnosis and control of the disease. Some later workers (Titze, *et al.*, 1923; Seelemann, 1923; Dahmen, 1923; Yamagiwa, *et al.*, 1931; and Campbell, 1938) obtained disappointing results with the agglutination test, particularly with chronic cases. Yamagiwa, *et al.*, frequently found strong agglutination reactions with the sera of normal cattle.

A slide flocculation test was developed by Priestley (1951), using Campbell's concentrated antigen, and this gave satisfactory results. Of 1,294 sera tested, 430 were positive with the complement fixation test, of which 3.02 per cent were negative with the flocculation test, whereas of 546 sera positive with the

flocculation test, 23.63 per cent were negative with the complement fixation test. Priestley concluded that the flocculation test was more sensitive than the complement fixation test: the specificity of the reaction was demonstrated when no positive reactions were found among 200 sera from English cattle.

In view of Priestley's findings, it was considered that a whole-blood, stained-antigen, rapid-slide test, on the lines of the pullorum test, might be possible, and if so, would answer many of our requirements for a field test.

The absence of a rapid field test has been a hindrance to the control of the disease in Nigeria. Pleuropneumonia is one of the chief problems in the cattle breeding industry of the Northern Provinces. Although the complement fixation test has been used with success for the control of pleuropneumonia in other countries, it has proved impracticable to use this test on a large scale in this country because of the nomadic nature of the herds, the great distances from the central laboratory, and the lack of skilled technicians. The work described was done in the hope that a simple rapid field test for contagious bovine pleuropneumonia might be developed.

Preparation of the Antigen

The antigen is based on that developed by Campbell (1938) for his complement fixation test. Khartoum strain KH 3/J of the pleuropneumonia organism is grown for ten days at 37° C. in Bennett's broth, (Bennett, 1932), supplemented by the addition of 0.25 per cent glycerol. A motorised Lister cream separator, revolving at a speed of 9,200 r.p.m. (R.C.F. value 4,780) may be used to separate the organism from the culture medium. After passing the culture twice through the separator at a rate of approximately 50 ml./min., the separator bowl is removed, and the deposited organisms washed off the interior with glass-distilled water. The suspension is standardised to twice the opacity of Brown's standard tube number 10, as measured on a photo-electric nephelometer and placed in a water-bath at 100° C. for ten minutes, cooled, and then transferred to a ball mill containing small glass beads (2 mm. diam.). The resulting particle size must be sufficiently small to make a stable suspension only, as prolonged grinding may reduce the floccular particle size in the test. The suspension is rendered isotonic by the addition of 0.85 gm. sodium chloride per 100 ml., and 0.25 per cent phenol added as a preservative. An aqueous solution of methyl violet is added to a final concentration of 0.01 per cent and the antigen is then left overnight in the refrigerator to allow it to take up the stain; 0.2 per cent of sodium citrate is dissolved in the suspension to prevent clotting during the test.

Each batch must be tested with known positive and negative sera, as antigens vary in sensitivity; auto-agglutination may occur, and samples ground too long in the ball mill may not be agglutinated. One sample, (not prepared by us) which although of inferior quality for complement fixation tests proved over-sensitive for agglutination, and gave positive reactions with the sera of a number of vaccinated animals which had been negative for several weeks.

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Technique

Rapid Slide Test.

A drop of blood is removed from the ear of the suspect beast, and transferred to a white tile. One drop of the stained antigen is added from a dropping pipette, The two drops are mixed with a platinum loop, and the plate rocked slowly to facilitate mixing. The results are recorded on an arbitary scale from + to +++ depending on the nature and time taken for the floccules to form.

Complement Fixation Test.

The complement fixation test used for comparison was carried out according to the technique described by Campbell and Turner (1936) and Campbell (1938), with minor modifications adapted from Hole and Coombs (1947).

Preliminary Trial

Cattle at Maiduguri.

The pleuropneumonia quarantine area at Maiduguri offered an opportunity for carrying out the test on a large scale. Infected herds from all over the province are collected in this area for slaughter, and at the time of testing many animals had already died as a result of the infection.

The antigen was prepared as described, but at this juncture, no sodium citrate had been incorporated. The first 97 bloods were tested by the rapid-slide, whole-blood test, and checked by the agglutination and complement fixation tests on the sera. The results are given in Table I.

TABLE I

Whole-blood agglutination, serum agglutination, and complement fixation tests of cattle at Maiduguri

	Rapid-slide Test	Serum agglutination		Complement Fixation Test	
Whole blood		Positive	Negative	Positive	Negative
Negative ...	12	0	12 ...	0	12
Positive ...	85*	48	37 ...	40	45

* A large proportion of these may be false positives due to small clots, as an anticoagulant was not used in this test.

A comparison of the results obtained by the whole-blood test, and those obtained from the tests on sera, showed that a number of false positive reactions were obtained with whole-blood, which may have been due to clotting.

A correlation of post-mortem findings with the results of the serological tests could not be made, owing to the large number of atypical lesions encountered; only 28 out of the 66 beasts giving a positive reaction to the agglutination test showed typical lesions on autopsy, the remainder showing varying degrees of congestion and hepatisation. Facilities were not available for the attempted isolation of the causal organism from those cases not showing typical lesions, and no definite diagnosis was made.

The sera of 21 of the 28 beasts which gave a positive reaction and possessed typical lesions were tested by the slide agglutination and complement fixation tests; the lesions were sequestra only and the results of the tests are shown in **Table II.**

TABLE II

Examination of sera of 21 of 28 animals showing sequestra only. Comparison of rapid-slide agglutination and complement fixation

Rapid-slide Test (Serum agglutination)		Complement Fixation Test	
		Positive	Negative
Positive ...	17 ...	14	3
Negative ...	4 ...	1	3

Specificity of the Rapid Slide Test

Rapid slide tests were carried out on two herds of cattle which had had no contact with pleuropneumonia infection for several years. Of 102 beasts tested none were positive.

Kagoro Field Trial.

This trial, carried out on a recent outbreak of pleuropneumonia, involved 121 beasts; three had died of the disease and 118 were thus available for testing.

As a result of the experience gained in the preliminary trial at Maiduguri, 0.2 per cent sodium citrate was added to the antigen as an anticoagulant.

TABLE III

Field trial of cattle at Kagoro

Rapid-slide Test (Whole blood)		Complement Fixation Test	
		Positive	Negative
Number showing a positive reaction to the test	45	39	6
Those showing typical post-mortem lesions	32	30	2
Those showing atypical post-mortem lesions	12	9	3
Those showing no lesions at post-mortem	1	0	1
Number showing a negative reaction to the test	15	0	15
Those showing no lesions at post-mortem	3	0	3
Those showing atypical lesions ...	12	0	12
Those showing typical lesions ...	0	0	0

XUM

In Table III the findings are classified under three headings: typical, atypical and no lesions. Under typical only those cases showing marbling or sequestra in the lung were included. Atypical lesions included those showing various degrees of congestion or hepatisation. The results of the rapid slide test and complement fixation test are given.

Under the existing Ordinance, the whole herd of 118 were slaughtered. The rate of slaughter was governed by the limited local meat market. Owing to the short time available and the slow rate of slaughter, it was only possible to carry out critical autopsies on 60 of the beasts tested.

Tests on Vaccinated Animals

The present method of control of pleuropneumonia in Nigeria involves the vaccination of herds surrounding an outbreak. It was necessary therefore to ascertain for how long vaccinated animals were likely to give a positive reaction to the rapid slide test in order to minimise confusion between positive reactions due to vaccination and those due to the natural disease. In this experiment 29 cattle were used, including 14 controls. There was an even distribution of ages between the controls and the vaccinated animals; all of them were between one and three years old.

The vaccine used was a living Bennett's broth culture of the 88th generation of strain KH_3/J of the pleuropneumonia organism, administered in a 1 ml.

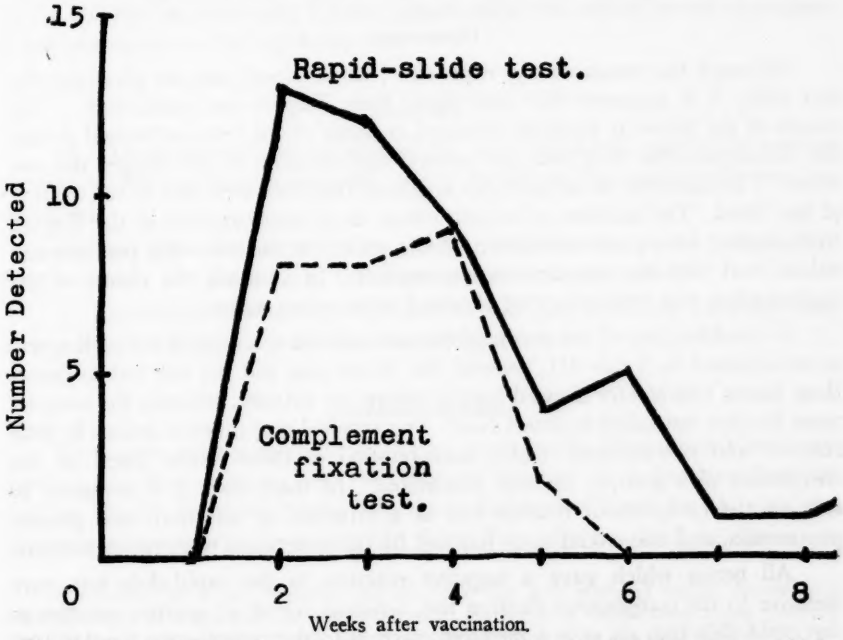

Fig. 1. Graph showing the number of beasts in which a serological response could be detected by the rapid slide and complement fixation tests after vaccination. (The total number of animals used was 15.)

dose subcutaneously behind the shoulder. This is the vaccine normally issued for use in the field.

Blood samples were taken at weekly intervals, and the serum submitted to both rapid-slide and complement fixation tests. The results are summarised in the graph, where the number of beasts in which a positive reaction was obtained each week by both tests is shown. Six weeks after vaccination the sera of all the vaccinated animals had become negative for complement fixation, but even after nine weeks two were still positive to the rapid-slide test. At this stage, the batch of antigen then in use was exhausted and the only other antigen available proved unduly sensitive, giving results that could not be correlated with those previously obtained. Observations on this group of animals therefore had to be discontinued, and we have not yet ascertained for how long a vaccinated animal may remain positive to the rapid-slide test. Two of the control animals were occasionally positive to the test, one slightly so on a single occasion and the other strongly so for three successive weeks. These have been excluded, as further evidence suggests that both these animals had been in contact with the disease before being purchased by the laboratory. One of them, subsequently used as a control when a batch of vaccinated animals was challenged by subcutaneous injection with virulent material, was solidly immune. The remaining twelve controls in the present experiment at no time showed any reaction to the rapid-slide test.

Discussion

Although the results of 157 rapid-slide, whole-blood tests are given for the two trials, it is apparent that only those from Kagoro are satisfactory. The nature of the floccular particles obtained in many of the tests performed during the Maiduguri trial indicated that normal agglutination of the antigen did not occur. The character of the particles suggested that they were due to the clotting of the blood. The addition of sodium citrate as an anticoagulant in the Kagoro trial resulted in a good correlation of the results of the test with post-mortem lesions, and with the complement fixation test. In addition, the nature of the agglutination was similar to that obtained when using serum.

A consideration of the results of the whole-blood test carried out at Kagoro, as summarised in Table III, showed that in no case did the test fail to detect those beasts which later showed typical lesions on autopsy, whereas the complement fixation test failed to detect two. The atypical post-mortem lesions in both reactors and non-reactors render interpretation of these results based on the correlation with autopsy findings impossible. In these cases it is necessary to rely on the complement fixation test as a criterion of infection with pleuropneumonia, and this would seem justified by the experience of previous workers.

All beasts which gave a negative reaction to the rapid-slide test were negative to the complement fixation test, whereas out of 45 positive reactors to the rapid-slide test, six gave a negative reaction to the complement fixation test. In view of the fact that two out of these six negative reactors to the complement

XUM

fixation test had typical pleuropneumonia lesions, and that there were no reactors in 102 beasts with no contact with the disease, it is possible that the remaining four may also have been infected with pleuropneumonia.

Although the rapid-slide test has been carried out on relatively few animals, the results obtained indicate that it is as good as the complement fixation test, and may prove more sensitive; this agrees with the results obtained by Priestley (1951) with the flocculation test on serum.*

The results of the vaccination experiment showed that, although complement fixing antibodies were no longer detectable in the blood six weeks after vaccination, some of the animals showed detectable agglutinating antibodies for some time after that.

The main objection to the agglutination test raised by previous workers is its failure to detect chronic cases, i.e., when a sequestrum is the only lesion. As will be seen from Table II, the rapid-slide test failed to detect four only out of 21 chronic cases, compared with six by the complement fixation test; of these chronic cases, three were not detected by either method and probably carried sterile lesions only. From this we conclude that the rapid-slide test is at least as reliable in chronic cases as the complement fixation test.

The failure of Campbell (1938) to detect chronic cases may have been due to the fact that he was using a tube agglutination test, which in our experience is not as sensitive as the rapid-slide test.

Summary

(1) The preparation of an antigen for a whole-blood, rapid-slide test for contagious bovine pleuropneumonia and a technique for making the test are described.

(2) This test is considered to be at least as satisfactory as the complement fixation test for the diagnosis of the disease, even in chronic cases.

(3) Agglutinins persisted in the blood of vaccinated animals for some time after complement fixing antibodies had disappeared.

Acknowledgments

We wish to acknowledge the assistance given by the Director of Veterinary Services, Northern Region, and the Assistant Director of Laboratory Services and Mr. R. M. Gambles, Senior Veterinary Research Officer, for carrying out the complement fixation tests, and Mr. R. C. Early, for technical assistance.

* Exceptions can occur, however, and one beast has been observed which, following a challenge with virulent material subcutaneously, developed a complement fixing titre of 1 in 160 and a week later 1 in 640 and yet remained negative to the rapid-slide test. The following week the serum had a titre of 1 in 1,280 and gave a ++ reaction to the rapid-slide test.

We are grateful to the Inspector-General of Animal Health Services, Nigeria, for permission to publish this report.

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THE EXPORT OF ARAB HORSES TO INDIA IN THE 14th CENTURY

By R. H. A. MERLEN, M.R.C.V.S.

IBN BATTUTA, the Mahommedan Marco Polo, spent the middle years of the 14th century travelling extensively into almost every part of the Old World and visiting Africa, Southern Russia, India, China and South-east Asia. Everything was of interest to him—politics, archæology, natural history and agriculture—and his most interesting comments on what he saw and heard during his twenty-four years of wandering are recorded in the book of his travels, the "Tuhfat en-Nuzzar."

It is of considerable interest to notice that when speaking of the south-western corner of Arabia, the Yemen, he makes the following observation: "They send a good type of horse from here over to India and make the passage across in one month if the wind is fair": by the way he speaks it would seem as if this export of Arab horses direct to India was a well-established traffic in the early thirteen-hundreds and that it took place annually at the blowing of the monsoon, the horses being presumably ferried over in open dhows. Large areas of India were, of course, under Muslim domination at this time but even so the demand for horses from Arabia cannot have arisen entirely from a desire to promote home industries: moreover, the adjective applied to them—"itaq-generosus"—seems to indicate that they were valued much above other horses.

The importation of these horses resulted in all probability in the mingling of Arab blood with that of the local breeds and in the production of Arab characteristics among the indigenous animals: it is essential, therefore, to bear

the existence of this trade in mind for otherwise it is possible to come to very wrong conclusions when considering the history and the evolution of the horse in India.

ABSTRACT

FAMILIAL CEREBELLAR HYPOPLASIA IN CATS. Scheidy, S. F., *The North American Veterinarian*, Vol. 34, No. 2, pp. 118-119, February, 1953. 1 illus.

HYPOPLASIA OF THE CEREBELLUM is a rare abnormality in the cat. Only a few cases have so far been recorded (reference is given in the bibliography). The author has described three litter-mate cats (one male and two females) in which post-mortem examination revealed a marked symmetrical hypoplasia of the cerebellum. The symptoms such as asynergia-ataxia, muscular hypertonia and abnormalities of reflexes had been manifested shortly after birth. Macroscopically, the cerebellum in question represented approximately one-third to one-fourth of the size of this organ in a cat of equivalent age. Only the median and lateral lobes were noticeable. Serial sections were made for histological examination, but unfortunately the results of these studies have not been incorporated in the article. J.K.G.

REVIEWS

KNEEGA O LOSHADI (THE BOOK OF THE HORSE), edited by Professor S. M. Budienni. Moscow: Gosudarstviennoe Izdatielstvo Sielskokhozyaistviennoi Literaturi. Vol. I. Pp. 600. Price 17s. 6d. net.

PROFESSOR BUDIENNI is to be congratulated on the way in which he has collected and presented his great wealth of information and of which this book represents only a part—two other volumes being still in the press.

The first section deals with conformation and malformations, and is succeeded by a chapter on evolution and an account of the horse during the earlier historical periods. The better-known classical material is re-examined and there is some very interesting information on Siberian, Mongolian and Central Asiatic horses. The data has been gathered together from scattered and almost inaccessible sources.

It is, however, around the ensuing section, on breeds, that the greatest interest centres. Russia, and Asiatic Russia in particular, is a country of tremendous variation in climate and landscape. The effects of these various climates and surroundings on the horse and his development are of extraordinary interest, both from the narrower point of view of their effect on this one animal as well as from the wider consideration of the influence of climate on animals as a whole. We have the opportunity, and the profuse illustrations make the task an easy one, of comparing the product of the Trans-Baikal snows with the horse from the hot and arid plains of the Turkmen and Kazakhstan, of putting the little Yakutsk and Priobski ponies from the northern tundra beside the horses from Stavropol and the famous horses from the Don: on the one hand we have the trotters and the Arabs, and on the other the "tyazelovozi," the heavy draught horses with the massive Percheron crests; and in every case there are the fullest facts and figures on the breeding, the pedigrees, the sizes and the weights.

This is a work of importance to the veterinary surgeon, the physiologist and the horseman: its illustrations alone make it a well-worth-while purchase.

MANUAL OF INFERTILITY AND ARTIFICIAL INSEMINATION IN CATTLE, by P. G. Millar, M.R.C.V.S., and N. P. Ras. First Edition, pp. xvi + 340; 10 plates, 39 diagrams. London: Baillière, Tindall & Cox, Ltd., 1952. Price 25s.

SIR THOMAS DALLING, lately appointed Chief Veterinary Consultant to the Food & Agriculture Organisation, United Nations, in his foreword to this book expresses precisely the reviewer's opinion about the need for a book such as this, when he writes that "We welcome the publication of this textbook by two workers who have had much practical experience of the subject."

The manual, essentially practical in its scope, is divided into four sections. Section I deals with the reproductive anatomy and physiology of the bull and cow, describes a suitable procedure for the clinical examination of the stud bull, and outlines the standard methods for evaluation of semen. Section II reviews the various ætiological factors responsible for infertility under the following headings: Genetic and Developmental Factors, Infective Factors, and Various Environmental Factors.

Section III sets forth a detailed consideration of infertility as it affects the individual animal, and according to the anatomical structures involved. It also includes a chapter on the important subject of infertility considered as a herd problem. Lastly, Section IV summarises the methods and techniques of artificial insemination.

The illustrations, which have been specially drawn make clear relevant points in the text.

There is no bibliography to accompany the textual matter. The value of the work would have been greatly enhanced if references to the original papers

XUM

consulted by the authors had been included. Despite this criticism, the authors should be commended for the attempt to assemble the rather widely scattered information relating to infertility in cattle.

DICTIONARY OF SCIENTIFIC TERMS: Pronunciation, derivation, and definition of terms in Biology, Botany, Zoology, Anatomy, Cytology, Genetics, Embryology, Physiology. By I. F. Henderson, M.A., and W. D. Henderson, M.A., B.Sc., Ph.D., F.R.S.E. Edinburgh: Oliver and Boyd Ltd., 1953. Fifth edition by John H. Kenneth, M.A., Ph.D., F.R.S.E. Pp. xvi + 506. Price 32s.

There is little need to speak of the popularity of a dictionary that has reached its fifth edition, especially as the previous one was published only four years ago. The present volume deals with more than thirteen thousand five hundred terms and although they are concerned mainly with the branches of biology enumerated in the sub-title, some terms in bacteriology and palæontology are included. The derivation and the definition of the terms are the satisfactory and valuable part of the dictionary and the pronunciation is, as admitted in the preface, the least satisfactory portion; it is subject to fashion and to continual fluctuations.

Students of comparative medicine and animal health, who want a reference dictionary for occasional service, will find the meaning and derivation of those words which may puzzle them when reading of some of the basic biological sciences. To those who are mainly interested in the derivation of the words, and want to understand the reasons for the use of many words in the subjects given, the book will be a constant source of interest and of information.

NEWS

The British Oil & Cake Mills, Ltd. announce that Dr. W. P. Blount is leaving their service in October in order to take up the post of Veterinary Director of Lederle Laboratories Division, Cyanamid Products Ltd.

He will be succeeded as Poultry Adviser to B.O.C.M. Ltd. by Mr. W. Carr, N.D.A., N.D.P., who will also take over responsibility for B.O.C.M. development work in pig and poultry nutrition at their Experimental Stations at Stoke Mandeville and Selby.

It has been arranged that Dr. W. P. Blount will continue to act as consultant to B.O.C.M. Ltd. on the veterinary aspects of animal nutrition.

NOTICE

The Distillers Company (Biochemicals) Limited announces that "Distafeed" Penicillin Supplement, in containers of 1 lb. (2s. 10d.), 10 lb. (25s.) and 50 lb. (108s. 4d.) is now freely available for sale without prescription. Orders should be sent to wholesalers, and not direct to the manufacturer.

They also announce the introduction of "Streptaquaine" brand Solution of streptomycin sulphate. This product presents streptomycin in a ready prepared stabilised solution, intended for intramuscular injection without further dilution.

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"Streptaquaine" Solution costs the same as streptomycin.

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